Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Hello everyone, welcome back to the NSCCI in the rodent module of the rodent neurosurgical aspects. So, most importantly, if your study involves animal models and small animal models to prove whatever you are studying in the neural engineering aspect. In the last few sessions, we have covered all the equipment that is required and the drugs that are involved, how to select those rats for anaesthesia, and what sort of monitoring needs to be done before you subject the rat to anaesthesia. So, now that the rat has been anaesthetized, during the process of surgery or anaesthesia, what are the parameters that need to be monitored to make sure the survival rate is good so that your surgical outcome is good and you are trying to get the expected data? In neural experiments, especially, the brain function must be maintained as it was before your experiment. So, this healthier outcome and a good neural outcome after all the experimental procedures that you subject the rat to, and then you are going to implant the various materials into the brain or the spinal cord.

The data that you are going to collect needs to be optimal and that is going to be dependent on the health status of the rat. For example, during the procedure, if there is some hypoxic injury or there is a stroke caused by the anaesthesia or by the direct injury from the surgery, all your results are going to be unreliable. Not only that, the entire procedure has to be repeated on a different subject. So, it is not only the rat's life that you are going to lose, but also your precious time and effort that you have taken will be wasted if the monitoring is not done up to the mark, alright. So, with that introduction, we will see what are the parameters that need to be monitored—basic parameters—and as I said, this is going to be a primer; you need to spend a little more time with the introduction that I am giving and then get to know it. There is a significant learning curve with all this, but nowadays, the equipment and setup that we have already discussed are very user-friendly.

You will be able to use them right from the first day, but provided you know what those parameters are, what the principles in maintaining those parameters are, what physiological functions you need to observe, and a little bit about the theoretical aspect of those physiological parameters will be discussed today, alright. So, we have covered all these topics in rodent anaesthesia in the last two sessions. So, today's entire session is going to be entirely on vital monitoring and postoperative care, which is very important

and that is what is going to decide the outcome, alright. So, let us look at how there is an impact on the physiological status of the animal. For non-biological students, the word physiology refers to the functional status, alright.

So, functional—the word functional—becomes a little superficial, and physiology refers to the entire system physiology. For example, there is cardiac physiology, respiratory physiology, and neurophysiology. So, these are all terminologies that refer to the functional status of each body system, alright. So, here is an example where the anaesthetic agent has an impact on the heart rate. This is just one example.

Of course, other vitals like respiratory and temperature are also going to get affected and we will see how those are monitored. So, I have taken an example where the anaesthetic agent has a direct effect on the heart rate. As I had explained earlier, the combination of ketamine and xylazine is going to have a direct effect on the heart rate which depresses it to around 55 per cent of its baseline heart rate, alright. That is how much it affects it. And of course, this is KX with isoflurane; another agent which causes 60 percent of it. Hence, if you are using an injectable anaesthetic agent, ketamine-xylazine is preferred, but the vital signs have to be monitored very tightly.

So, that it does not end up with bradycardia—that is, reduced heart rate—which will reduce the oxygenation to the brain by reducing the blood pressure. So, for non-biological students, I would just try to briefly summarize what all these parameters are that I am just trying to list out. The easy way to remember is TPR and BP. These are the four vitals that we all monitor even in humans, and the basic vitals that need to be monitored throughout a surgical procedure and any parameter that we discuss is directly or indirectly related to these four parameters, alright. So, T stands for temperature, P stands for pulse, R stands for respiratory rate, and BP stands for blood pressure, obviously, alright.

So, here if you want to divide the body systems, then the pulse and BP refer to the cardiac system, alright, and respiratory rate is the respiratory system, alright, and temperature is an overall function, but ultimately controlled by the brain itself, alright. So, you need to remember temperature falls because the basic metabolic function of the rodent's body comes down, alright. So, the BMR comes down. So, the heat generated in the rodent's body will also come down, not only that you are blunting the CNS, but the central nervous system, which will control the overall temperature will also come down. So, generally, it is a hypothermic state, alright.

So, all the critical functions in the body, especially the enzymatic functions, need a temperature of around 37 degrees Celsius, right? So, this is tightly controlled in a very narrow range wherein any higher temperature of hyperthermia or lower temperature of hypothermia both are detrimental to life; that is very, very important to remember, alright.

So, next is the pulse and blood pressure; obviously, those refer to the cardiac pump activity, alright. So, since I am discussing the parameters, I might as well cover the entire parameters here wherein I am introducing a lot of terminologies and it is important to pay attention and remember all these. So, in rats, the heart rate range is around 250 to 500 beats per minute, which is a pretty wide range.

However, that should be the range wherein you are monitoring the heart rate with a respiratory rate of around 36 to 50 per minute. Ok, respiratory rate is going to be 36 to 50 per minute. So, these are very important numbers to remember and many systems will automatically display these parameters and not only display them, it will also raise an alarm if the threshold has been set manually within their systems. Alright. So, the cardiac pump is very important to maintain the blood pressure which will maintain the cerebral perfusion pressure. Alright. So, if the blood pressure falls or the heart rate falls, the blood pressure will fall. When the blood pressure falls, the cerebral perfusion pressure will fall, wherein the oxygenation and metabolites delivery will come down to the brain and it leads to what is known as hypoxic ischemic injury to the brain. Alright. I am trying to introduce a lot of terminologies here, but every single one of them is very, very important to remember to understand the importance of this vital monitoring.

So, hypoxia refers to reduced oxygenation, ischemia refers to the reduced blood supply in general. Alright. So, what leads to hypoxia? This can be many wherein oxygen saturation itself will come down, which we will discuss in detail. Ischemia is obviously when the blood pressure falls and the heart rate falls. Alright. All these are intertwined and it becomes a complex physiology, but then easy to remember if you can at least recollect TPR and BP. The best way is to have a good monitoring system that will not only help you with the alarms that are happening in a very dynamic range, but also tell you when to stop the surgery, and when to abandon the surgery to save the rat's life so that you can at least repeat it later. It also tells you various side effects of the drugs that you are using so at least for the next experiment you can improvise. So, as I said, the animal should be continuously monitored during the anaesthesia with an assistant dedicated to the monitoring.

It is not that the operating lab person is going to do everything; you should have help to assist with the vital monitoring. And the important thing, as I said, is the respiratory rate and the character. The most shallow respiration indicates the lightening of the anaesthetic plane; deep breaths indicate deepening. This is one of the reasons, alright, for the change in the respiratory rate and character. Alright. So, the lightening of the anaesthetic plane is that the rat is becoming more and more conscious.

If you remember the plane of anaesthesia that we discussed based on the minimal level of concentration, if your dial setting on the vaporizer is on the lower limit, the percentage of the anaesthetic gas is also on the lower limit, and then the plane of anaesthesia is going to be lower. That is either the loss of memory plane or you know, loss of consciousness state which is superficially just sedated, not unconscious. So, it is very important to look at the anaesthetic plane which will be indicated directly either by the MAC or the dial setting that is being given and if you can monitor the respiratory rate of the rat. So, another important thing is hypothermia. As I said, this will prolong the recovery and many times it will not recover completely and then spiral down to death.

If there are additional components of acidosis and coagulation disorder coagulopathy. Alright. So, this we will discuss in detail later, but temperature is a very important parameter hence for the reason that I just discussed, you need to provide additional heat at all times when anaesthetized and in the immediate recovery period. That is very important. It is not just enough to give the temperature, you know, the heating atmosphere or the additional heat only during the surgery. You must carry it down to the recovery period until a full recovery happens. Alright. So, the reflexes; this is a very important parameter. Absence of a pedal withdrawal reflex.

If you all remember the illustration I did and I said when you pinch, there is a withdrawal of the hind paw that is happening. This is the most reliable indication of the attainment of a surgical plane of anaesthesia in rodents because you are giving pain and you are looking for a lack of motor response. Ok. The basic requirement is the rat should not move when you cut the skin. Ok. So, when this pedal withdrawal reflex is attained, that is what you are going to see, but at the same time, it should not be so deep that it will affect the autonomic reflexes as I just discussed in the last few slides. Alright. So, it is always good to have such a rodent anaesthesiarecord which will also have various details about the surgery. Ok.

You can include all the identification details of the surgeon, the animal's ID, the procedure, and whatnot. Along with that, you give a detailed description of the actual anaesthesia being used. The cocktail being used, what exactly is the mixture that you are using? If it is ketamine or xylazine, what are the dosages of the same, how much is the volume that you are going to inject, and what time did you put the rat for induction? So, time is very, very important because as the time prolongs, the recovery is also prolonged. So, you can sort of predict the recovery time based on your entire duration of anaesthesia and then it is also nice to use preemptive anaesthesia.

As I said, to blunt the autonomic reflexes in the sense a response of heart rate that is tachycardia and a sudden rise of BP is unwarranted when you make an incision. There is an additional amount of pain given to the rat. So, to blunt that you can infiltrate the local anaesthesia-like lidocaine or buprenorphine to blunt that effect due to the pain. So, that

the vital parameters stay smooth throughout the procedure. And of course, you can also include the details on surgical preparation in the same record.

So, the most important time thing is when the surgery begins whether is there a withdrawal reflex or not and how is the mucous membrane seen. So, as I said that will give us an indication of the health status of the rat which is, sorry. So, it depends on the colour of the mucous membrane. This was discussed in the last session. Generally, pink is very healthy, pale is anaemic, and blue is cyanosis. Alright.

So, cyanosis is due to, again, reduced oxygenation. So, there is something wrong before you even begin the procedure and you have to abandon the procedure and look at the cause of this cyanosis. Pale is obvious that the rat is anaemic. Alright. So, these are the most important parameters that you are going to document every 5 minutes. Sorry, 5 minutes. It can go as long as the surgeries. So, you need to document at 5 minutes, 10 minutes, 15 minutes, and the parameters that are used are how much oxygen is being used; that is very important.

And then the percentage of isoflurane that is being used, what is happening to the respiratory rate, how is the mucous membrane during the surgery, when the surgery ends, and is the animal awake or not. And if there are any untoward incidents during the anaesthesia or surgery, that also needs to be documented because all these are going to have a bearing on the outcome. Surgical outcome or the functional outcome if you are doing the chronic study. Alright. And then, of course, some details about the postoperative analgesic care, whether you are going to use isotonic saline or you are going to use the analgesic agent and if yes, what route. And then a little bit about where did the animal recover and how was the recovery. Alright.

So, and then the heating source whether it is there or not. So, it is a sort of checklist that you are trying to maintain along with the monitoring which is very, very important because you are trying to look at the huge anaesthetic responsibility and there is a surgical responsibility going on; you need to set up the entire apparatus. So, I would strongly recommend that you have a checklist, have a written protocol stuck on your lab wall wherein you know every step you are using until it becomes automatic for you. It is better to have, I would say, a checklist is a very important aspect even in human endeavours and it is more so important in human endeavours. It is a good habit to maintain a checklist and have such an anaesthetic and surgical record. Alright.

So, now let us look at what are the apparatus that are available in the market to use for rodent anaesthesia and surgery. So, this is one such a very good setup where there is a heating pad built into the system and then there are various, you know, gadgets and peripherals which are used to monitor respiratory rate, cardiac rate, and end-tidal carbon

dioxide and whatnot. So, how are we going to use it? So, as I said these are the broad physiological systems that can be monitored with various... This is one of the examples by the Cato group where the modular physiological monitoring uses a suite, is available with a physio suite setup where they have homeothermic warming with the right temperature peripheral and there is most a modular system where heart rate and pulse oximetry. Alright. I am sure post-COVID all of you are familiar with this pulse oximetry which can be applied onto the paw wherein it shows SpO2. Alright. SpO2 is oxygen saturation which needs to be at least more than 95 percent and then there are ventilatory parameters checks and of course, end-tidal carbon dioxide monitoring.

If the $ETCO_2$ is high, it means there is carbon dioxide retention happening in the respiratory system. So, you will need to look into the plane of anaesthesia and check the respiratory rate to see if it is deep or shallow. All this has to be monitored when it gives an alarm regarding various parameters. There is infrared warming along with the system to maintain the temperature, and the probes that can be used are rectal probes for the mice and rats. This is a very good peripheral or accessory device to monitor the core body temperature.

You can even pre-program it to the set temperature, and then there is automatic feedback control, where the warming increases if the temperature is falling. This is a very good setup. If that is not available, you can even manually increase the temperature. MOSDAT is dedicated to mice and rats; there are power sensors that detect the heart rate and pulse oximeter, and it also has the option for monitoring the respiratory rate along with automatic feedback control.

Ventilation parameters are very important to look at. The ventilation can be controlled by pressure or volume, which decides the delivery of the gas. This is automated based on pressure and volume control. I suggest you do a more detailed study on these pressure and volume controls of the anaesthetic agent, which will ensure a very good plane of anaesthesia and smooth anaesthesia throughout the procedure. Capnoscian is another accessory that samples carbon dioxide in the entire volume. Any retention of increased carbon dioxide indicates reduced gas exchange or increased catabolism, leading to hypercarbia. Hypercarbia is the condition where there is accompanying hypoxia—oxygen is reduced, and carbon dioxide is increased. This leads to acidosis, specifically respiratory acidosis.

The discussion on acidosis, homeostasis, and electrolyte imbalance is beyond the scope of the current module, but I hope you read a bit about all the principles involved in the physiological mechanisms of acidosis so you can correct them if such instances occur. After anaesthesia, how do we care for these rats? There must be continuous care until they make a full recovery. The animal should be observed continuously until the righting reflex has returned. The righting reflex is when a rat that is placed on its back

automatically returns to a normal position. If it stays in the same position in the cage, it means the righting reflex has not come back. These are important postural reflexes that give an indirect indication of the status of the central nervous system.

The animal should be returned to a warm, draft-free cage that is placed on a warming pad or under a heating lamp. Temperature is again important in the recovery aspect, not only during the surgery. Animals should not be put together, as they are likely to walk around and even trample others when they are at different stages of recovery. It is better to return them to social housing as soon as possible once the rat has made a full recovery. If the surgery has been performed, the bedding should not stick to the wound. This is very important to prevent infection and trauma, as the wound is raw.

It is better to use paper rather than wood shavings or bedding for the postoperative cage. Ensure that the animal can reach water and food sources. If not, place them on the cage floor or consider administering fluids to prevent dehydration. Various supplements are available if you think the food and water intake is not adequate. You need to monitor the animal's basic biological functions, including food and water intake, body weight, and urination. All this will provide clues about the health status of the rat following the procedure for the next 2 to 3 days.

If there are any clinical signs of distress, they need to be monitored daily for at least the first week following surgery. This recovery time is 7 days, which is why many experiments allow a 7-day gap if multiple implants or surgeries are involved. After every surgery, you need to allow 7 days for the physiological system to reset itself to its initial stage. During these 7 days, monitor the following parameters.

After surgery, there must be an analysis plan to ensure that the rat is not in distress, as distress affects full recovery and makes the rat more irritable. If you plan to do any experimental behaviour, it will not be cooperative. To assess the post-procedural status of the rat, a score of 6 can be given if it is very active, curious, and fast, with occasional breaks. As the activity decreases, the scoring goes down. This is a very important stage to recognize.

The rat will be uninterested in its surroundings, rarely active, mostly sleepy, and have reduced food intake. This is an alarming state; unless you identify and rectify it to bring it to a 5 or 6, it will likely deteriorate quickly. A moribund state indicates an almost dead rat, where death is anticipated if there are breathing issues and no activity. Such scoring helps monitor the health status after surgery and provides a chance to rectify it at the earliest.

Others are signs that this is a detailed scoring where you can check the body weight and give the scoring, and you can check the breathing pattern, mobility, and reaction to

stimuli. Check the coat to see if it is ruffled and not clean. Generally, the rat keeps grooming itself so that its fur coat is smooth and shiny. If it is not clean and ruffled, it indirectly indicates that the rat is not active. Of course, posture and behaviour—social behaviour, apathy, and hunched posture—are very poor indicators and also suggest that the rat is in a very poor state. This should be checked to determine what is causing this sort of posture and social behaviour.

Feces also give us an indication of sepsis. If there is an infection, there will be diarrhoea and the stool will start becoming soft. Crusting of the orifices will give an indirect indication of dehydration. Summarize these observations and have a total score. Monitor this daily with date and time.

This monitoring should be done for the next 7 days. All these parameters need to be monitored and scored, and improvement should be noted over the next 7 days. A rodent card can be maintained in the recovery cage to help with a quick look at the analysis and to determine whether the behaviour is normal. This card should be signed regularly. Hydrating gels are commercially available and are very important for hydrating the rat following surgery. If the feeding and drinking habits are not adequate, perform the skin tenting test—if the tent persists, it means the rat is dehydrated.

Maintaining hydration using hydrating gels is crucial for providing the extra hydration required. As a last session, we will briefly touch upon local anaesthesia. I am pretty sure all of you are familiar with this already. Local anaesthesia blocks the nerve impulses locally by specifically binding to the voltage-gated sodium channels in the nerve cell membrane. If that is the neuron, dendrite, soma, axon, and axonal terminals.

It acts on the sodium channels and blocks the activity of the nerve cell. Usually, the route of administration is topical to mucous membranes or injected directly into the tissues and around the nerve bundles. Local nerve infiltration depends on whether there are nerves that can be blocked. If there are no nerves that can be blocked, then all areas supplied by that particular nerve will be blocked. These are called nerve blocks or regional nerve blocks. If it is directly infiltrated into muscular or subcutaneous tissue and an incision is made, the anaesthesia is provided only to the depth of that particular plane that has been infiltrated.

Administering local anaesthesia before a painful stimulus is considered an adjunct analgesic to opioid or NSAID analgesics. These can be used together to provide good pain relief. Using it as a primary analgesic is discouraged due to its shorter duration. It is always used in conjunction with either general anaesthesia or opioid and NSAID analgesics to maintain analgesia. It has a quick onset of around 1 to 2 minutes but is short-acting. Lidocaine has a quick onset and is short-acting.

Bupivacaine has a slow onset but is long-acting. Usually, these are mixed for the principle of induction and maintenance. When used in combination, as mentioned, lidocaine plus bupivacaine in the same syringe can provide fast onset with a relatively long duration of action. This is a popular mixture, similar to the ketamine and xylazine combination. For rodent use, you need to dilute 1 to 2 per cent lidocaine to 0.5 per cent and 0.5 per cent bupivacaine to 0.25 per cent total for feasible volumes and infuse it at the incision site. This can be used for a scalp block. These are popular mixtures for long procedures and long anaesthesia if the surgery is within 5 to 10 minutes of the procedure.

What has been shown here on the right-hand side is the nerve block, where the nerve supplying the skin around the eyes is blocked for any procedures performed around the eyes. This is just an example where a specific nerve can be blocked using local anaesthesia.

This brings us to the end of today's session. To summarize, we have covered all the equipment, monitoring parameters, and drugs involved, and discussed the options for injectable and inhalation anaesthesia, as well as monitoring during surgery and anaesthesia. This provides a nutshell view of the entire workflow of anaesthesia and surgery. I strongly recommend that you read more about the aspects covered. I have tried to cover most of the principles involved, but there are many more details to explore and improvisations needed to customize it for your particular experiment. In the next few sessions, we will deal with general animal handling and how euthanasia is performed after surgery if you are trying to harvest the brain.

These topics will be covered in the next few modules. Thank you all for your kind attention.