Lec 09 - Principles of Stereotactic Rodent MicroNeurosurgery

Hello everyone. So, we are continuing with the module of Rodent Microneural Surgery. So, the in the previous few lectures we covered human neuroanatomy, comparative neuroanatomy and the most important section of rodent neuroanatomy as well. So, since we have a fair amount of idea on the neuroanatomy from now on we will be sort of applying that knowledge to achieve a little more knowledge on the neurosurgical aspects for stereotactic apparatus and the knowledge for using the stereotactic apparatus for the surgical sake. So, then how do we use the serotactic coordinates, how do we calculate a particular target, what are the terms and you know various target sites that are available for various neural researches. So, let us go through the stereotactic setup as such.

So, this is the outline for today's lecture. So, we will be covering the various anatomical planes because those terminologies are very important because time and again you will read through literature and you will end up in communicating with others. So, knowledge about the good knowledge about anatomical planes, anatomical orientation because in neurosurgery the very important aspect is that you are not exposing the entire structure like for example, if it is an abdominal surgery or for that matter a chest surgery, you will be exposing the entire organ in situ. But when it comes to brain surgery you are targeting a very small area or you are making a small drill hole and you will be targeting a very deep structures.

So, here there is entire setup of stereotaxy comes in. So, what exactly is the stereo refers to the three-dimensional space, tactic refers to the touch or the tactic refers to the entire three dimensional space that will be used. So, this stereotaxy is very special for a neural experiments you understand. So, sorry for that. So, we are covering the anatomical planes first, then we will go ahead with introduction to the serotactic setup.

It is very important to understand this equipment and it is a very versatile equipment to use for most of the neural surgeries and how to use that setup, how do you sort of you know troubleshoot if something goes wrong and then you also need to know the various skull anatomical landmarks and variations. So, this section has already been covered in the previous neuroanatomy, but today will be more about applied anatomy how we are going to use that particular knowledge for stereotactic surgeries. So, the next thing is the stereotactic principles and coordinates. So, from now on we are going to deal with lot of the stereotactic surgeries and stereotactic targets, but it is very important to understand the principles behind it. Unless we know the principles though you have various sources like JOVI or for that matter various atlases that are available for surgeries, even though you can replicate most of it, but if something goes wrong you need to know how to troubleshoot it or how to modify it and you will be able to modify that particular

protocol for your experiments if you can understand the principles behind it and understand how these coordinates are developed and how these will be applied.

So, that is very important. And next I will show some of the examples of how to set up a particular stereotactic target. When it comes to the plane we need to understand the XYZ axis in the rodent body. So, all of you are familiar most of you will be familiar with the Cartesian coordinates. So, it is more or less we have taken that theory of Cartesian coordinates and applied to the stereotactic space around the rodent,s head.

So, but before we go ahead with the details of the stereotactic scene one what you need to understand is the how the X and Y, XY and Z axis are oriented for the rodent body because it is the foreleg animal and because of the position of the you know body naturally these are the axis that will be considered vertical axis, horizontal axis and transverse axis. When it when we apply those axis these are the anatomical planes of orientation. So, one can see here as the dorsal or superior that is a terminology that needs to be used to indicate any structures on back of the rat ok. When it comes to the under the belly that location is called ventral or inferior. Inferior and superior is a common notation, dorsal and ventral are the anatomical terminologies that are used.

So, this I did make a passing mention in previous lecture on anatomy what is known as anterior and posterior for front and back. We do not use the term front and back we generally use anterior and posterior. So, what you need to understand is rostral and caudal. So, that particular word is used if it is towards the mouth and something closer to the nose in that position that we you would say rostral anything goes towards the tail you would generally use the word caudal. Because definitely rat I mean at least the rat which is alive it would not be you know still or you know alive rat is more it makes more sense to use the word rostral and caudal, but it is almost synonymous with the term anterior and posterior and something known as right lateral and left lateral.

So, lateral refers to the side of the rat. So, the right side is right lateral and the left lateral. So, this is important in the head what so, on the side is lateral and on the under the brain is ventral surface, the superior surface or the top of the brain is dorsal surface. The front portion of the brain would be anterior or rostral, the back portion of the brain would be posterior or caudal. So, this orientation is very very vital and we will be using back and forth such terminologies to explain various you know the relations of the targets.

So, when it comes to that this particular terminology is also very vital to remember and understand. One is the sagittal plane and most of the biology students or people will be familiar with these sections with you know these points. So, sagittal section is something

which goes right in the midline. So, I had used these terminology in the previous lecture as well. So, sagittal plane goes through the middle and even the you know the this plane can move laterally to the left or to the right and, but then that section is called sagittal section.

So, this is the very important in histopathology or even for that matter the stereotactic atlas which we will be discussing very soon. So, this is you know sagittal section, this is what it means. It goes through the midline of the brain or the body and then it goes along the sides. So, when it moves to the left then the section will be to the left lateral and then this is how it goes to looks like from front to back the entire structure will be seen. And you see any target that needs to be targeted there which I will be discussing again the stereotactic targets.

So, let me explain how it would look like. So, let us see we select a target there ok. So, the point of entry should will be from there all right. So, that is the importance of the sagittal section. Whenever we discuss a stereotactic coordinates you need to know three dimensionally in all three planes.

This is the sagittal plane, the next plane that we discuss is the coronal plane and the axial plane. In all three planes you need to know the structures in and around the target that you have chosen. And even in histopathology it is very important to look at all three sections. So, this is the sagittal section wherein you can see from front to back all the structure that are there front to back in this particular plane. One can continue the slicing this way which is going to be the left lateral or you can continue the slicing right lateral.

But every single slice will be oriented this way wherein this is anterior that is posterior. So, it is I mean if at all you have only this much of brain which you have harvested from the rodent then it is important to tell the you know the processing lab what is front and what is back especially when you are only you are sending only this much. If it is a sagittal section and if they are slicing it then that problem is avoided, but then very very important to orient that particular specimen which you are sending it for histopathology. So, that is when these terminals makes a lot of difference not just there even here when you are discussing the stereotactic surgery, when you are discussing the stereotactic catalyst while choosing the target is very important to see whether are you dealing with the sagittal plane or are you dealing with the coronal plane all right. So, that is about the sagittal plane.

Moving on to the frontal section or one can say that this is the coronal plane. So, this is the coronal orientation wherein is perpendicular to the sagittal plane here that was the sagittal plane what is perpendicular to that plane is coronal plane or you can say frontal

plane all right. So, the section goes like that all right. So, then that is how the histopathology would look like wherein that is the midline all right then this is right lateral that is left lateral. So, you will see the width of the entire brain ok.

What you saw earlier was a length of the brain this would be the width of the brain. So, one need to be familiar where this section is taken. For example, if it goes only through these olfactory tubercle will be only a small olive kind of a section. So, I will be dealing with the atlas further again and if it goes only through the cerebellum you will not see any of the cerebral cortex. So, it is very important to know where exactly the coronal slice is taken and their atlas which I am going to discuss is of very handy very helpful to orient yourself to know which was your target which target did you even use it to develop the coordinates and from where the slices have been taken.

So, that important it is. So, this is the coronal plane and the next one is the axial plane or horizontal. So, I use the word axial here previous terminology was sagittal second one was coronal the third one is axial. So, if you look at it. So, this will be coronal that will be the axial plane and that will be the sagittal plane which are perpendicular to each other. So, axial plane again goes from front to back all right, but it taking parallel to the floor the slices will be parallel to the floor from below upwards another slices goes like that.

So, you will see the structure right from front to back and then you will also see the width of the brain and if suppose that is a target you need to see this target in axial plane coronal and sagittal plane that is the third plane that we are discussing. So, once you are familiar with the anatomical orientation what we need to know here is the stereotactic setup. So, this is the stereotactic setup which is from the various companies are dealing with the most standard will be the RWD and then there is Soaring there are many other companies which deals with this sort of setup. This is a very basic stereotactic apparatus nevertheless very versatile which can be used for every neural experiments. So, let me introduce briefly the different parts of this stereotactic setup.

You will have to sort of get familiarized very well with this setup before you actually start performing the experiment. So, I suggest if you have this apparatus please test it out before you start the animal experiment. There are few things that you need to understand how to adjust this particular arm there, what these joints are and how these ear bars and nose bars are used. So, just to brief you I said there are sagittal plane and there is coronal plane and there is axial plane. So, these three planes will be targeted using these three arms here.

So, this is the instrument adapter all right here which is going to handle any sort of

implantation devices, any implantation, any surgery will be done through this particular adapter which can be you know we can swing it around and then brought it into the field. So, that is where the rat is going to be. We will discuss it in detail later, but just to introduce you to the various parts of this particular equipment that is the adapter which is going to hold various other various instruments. So, this particular adapter what shown here is basically for something like a needle or an electrode which is a depth electrode ok. This will also hold your syringes Hamilton syringes which is a different adapter and also a flat sensors or PCB which has a shank at the edge of it.

So, there are different types of adapters. So, that is the adapter for holding the instruments and that is the vertical vertically oriented axis wherein this height can be adjusted. So, this will give us the z coordinate ok. So, you will need to adjust x coordinate and y coordinates and z coordinate. So, this is for z while you twist and turn this it goes up and down for the z coordinate.

Then you have an x coordinate where wherein this adapter will move in this direction all right. So, then you have a y coordinate which to make the ah the adapter move in that direction which is the y axis. This is for the x coordinate that is for the z coordinate there. So, that these are the ah three ah adjustments that needs to be done and this will have a scale wherein you can take the coordinates and use those coordinates on to the scale adjust the scale. So, that a particular point within the rat brain will be targeted.

As to how we ah target that particular structure we will discuss it in detail ah in next few slides, but overall this these are the parts and the other important parts are ear bar. I guess let me clear this out. So, this is the ah ear bar here. So, this this goes into the ah ear of the the small animal that you are going to use for experiment. This one here is for the incisor bar and that is the nose bar.

The incisor bar the incisor teeth of the rat will go into this and this clip is going to be applied over the nose of the rat. So, it is a rigid fixation wherein the animal will be fixed in that way using these ah parts of the serotactic apparatus and this again can be moved up and down and there are instances where these needs to be adjusted which we will ah discuss in it in detail. So, as I said these are the three different axis with which it moves. So, as I said that particular ah axis will be adjusted with the ah you know the rotating head over there where the AP axis or the anteroposterior. AP is anteroposterior there again the terminology which we are using from the anatomical orientation.

So, and then as I said this is mediolateral which is the x axis. So, another that is another terminology that you all should be familiar familiar. So, if the if this is the different axis and that would be the exact midline of the rat orientation you know that is the sagittal

plane and let us say this is the coronal plane. We we are using a terminology called anterior and posterior. So, that is anteroposterior axis which is the sagittal axis and then you have a mediolateral in the coronal plane.

Anything closer to midline is medial anything away from the midline is lateral. So, that is another terminology that you all should be familiar. DV is dorsoventral when you move it upward it is called it is moving dorsally when you moving downwards it is moving ventrally all right. So, once the rat is fixed these three arms will be moved towards just the coordinates in these three axis which I just mentioned all right. Send to how to calculate those coordinates, how do you move it and how do you make sure that your adapter goes into the right position so that it will hit the target in question.

So, that we will discuss it in next few slides. There is a brief video as to how we use this manipulators here. So, I will just slow it down you can see that how one can swing the adapter and bring it. So, the idea behind getting this video is to give you a three-dimensional orientation this equipment is totally three-dimensional and unless you get your hands on this apparatus it is very difficult to understand every single bit that I am trying to explain. So, please understand the entire movements all the joints all the arms.

So, it has a double lead screw design where the axial movements are maintained. And if you have this particular additional equipment it will be very useful. This is a sort of an accessory to the basic setup that most of us have and this is a digital display of the coordinates which is very useful wherein you can see the coordinates as you move the move different arms there in various axes x y and z. Wherein the dorsal ventral movement will give you the different orientation depth at the in the z coordinate whereas, the medial lateral is 0. And this advantage of this is that when you clear it whatever coordinate that you consider I mean whatever point the reference point that you take will become 0.

Generally the reference point in the serotactic apparatus is the bregma. So, once you reach the bregma you clear all these coordinates and that becomes 0 and it is very easy to move forward from that 0 reference point. Because most of the literatures the reference atlases gives how far the coordinate is from the bregma because bregma is the reference point. So, there are two ways of calculating it.

So, we will discuss it further. So, this digital display actually sort of nullifies and gives you the 0 reference point from which you can easily move the arm in three different directions and achieve the coordinates that you are looking for all right. So, that is the display as you can see as they keep moving it moving the different arms in different

direction those coordinates are changing. So, that is the beauty of this digital display which is very useful if you can add it into the basic setup. Then that is the adjustment bar which I was talking to you about which is the nose bar. So, we can sort of adjust the vertical height as per the height of the skull that the you will be handling a small rare or small mice and some animals are bigger.

So, one need to make sure that your bregma is in line with the lambda. So, for that to happen this need these can be adjusted the nose bar can be adjusted. So, this I will be discussing it in detail. So, that is the ear bar and that is how it is removed and sort of adjusted and you need to make sure the exact midline orientation is maintained all right.

And this can also be moved back and forth. So, you can see that this the entire thing can be dismantled and cleaned and then you know arranged together again. But then if the as per the size of the animal one can move this nose bar and ear bar back and forth and adjust it and make sure that your fixation is rigid. Because you will be using the drill and you do not want that drill to slip because the animal head moves. So, one need to ensure a good rigid fixation with a good midline orientation all right. Only then your surgery there onwards goes smoothly because once the surgery is through and if the head slips your not only the experiment is going to get spalled it is life threatening for the rodent as well all right.

So, that is the importance of that and there is an additional accessory for stereotactic anaesthetic administration if you are using the inhalational anesthesia with anaesthesia mask. So, that is the stereotactic apparatus in general I mean we will discuss it further and I will let you all know how this apparatus is going to be used. Another important thing I need to highlight here is the vernier scale. I am pretty sure most of you will be familiar, but just to make sure that you know the coordinates are set well and the accuracy is entirely dependent on how you are going to use this vernier caliper. If you have a digital display that is going to help immensely and it avoids all these problems of vernier scale and looking at the markings.

If you do not have a digital display I would recommend that all of you get familiarize and you know get your hands on for these vernier scale usage. So, obviously, when you can see here there are two scales the primary scale is where the coordinate will be set and the decimal point is obviously, is going to be set on the vernier scale part of it. So, that these are the examples to you know get yourself acquainted for the use of vernier scale. The 14 will be on the primary scale, 0.7 will be counted on the vernier scale and adjust to the next graduation mark which aligns well with the primary scale.

So, these are the various examples wherein the primary and secondary graduation marks

are shown here and the coordinates are set. So, you will get these coordinates from the atlas directly or from the literature which has been used for that particular target. So, there are two sources one source of the coordinates are the literatures which has used similar target for their experiments, but one need to make sure that your species of the rat that you are going to use for those coordinates should be similar and of course, the body weight. Because as the body weight and the species changes there can be minor variations in the coordinates and the relations of these targets with the other brain structure and that can be really damaging for the animal. So, one need to ensure if you are taking the coordinates from the literature ensure that your species and the body weight matches even for that matter even if it is atlas you need to see which animal they have used to reference that atlas sections.

All those atlases are developed by making a serial sections. So, one atlas I can suggest I mean suggest and it is used mostly is the Paxinos and white atlas. So, this is I really appreciate this particular illustration and this is very very important to understand where in the cranium, where in the head of the rodent is exactly the brain is situated which is very vital. Because all you will be seeing in the initial part of the surgery is this you will only see the exposed part of the skull which is mostly here that is how much you are going to expose not more than that all right. So, then once you expose these are the various surface landmarks that are available one is Bregma the other one is Lambda. So, these are the very good reference points that needs to be used to calculate the coordinates and adjust the stereotactic setup for those coordinates then target the particular structure that you are doing the research on.

So, that is how the brain is situated within the cranium and in relation to the Bregma. So, the most of the rodents brain or the isocortics cerebral isocortics is situated between Bregma and Lambda. So, that is how vital it is and anterior to Bregma this is a small part of both primary motor cortex and secondary motor cortex are situated. So, but then I would say around 80 percent 80 to 90 percent of the brain is situated posterior to the Bregma all right. So, that is very important to know how and what part of the structure is in relation with the Calvary you know bone structures.

For example, that is the occipital bone which will encase the cerebellum. So, posterior to Lambda if your study is going to involve cerebellum all your coordinates are going to be posterior to Lambda all right. So, that is pretty important and if you are sort of targeting any structure under the surface of brain then the position of the rat would be sort of lateral position. You would not really target it from the top and that is important for all these stroke models wherein middle cerebral artery comes from below upwards. So, to target the main division you will be rotating the rat, fixing the rat in the lateral position that is on sidewards and make the incision between the ear and the eyes to

expose that part of the skull which is temporal bone and then target the middle cerebral artery.

So, that is how the correlation of the skull and the brain structures vital here to understand. So, this is the lateral position which I just described. So, this is the lateral surface that means, the side surface these of the rodent skull and if you see that is the junction of olfactory tubercle there and the rest of the brain and that is where it is situated all right and approximately that is Bregma and this is Lambda. We will be using lot of these term these lot of slides will use these Bregma and Lambda and for that matter the entire neural experiment is based on these reference points. So, I suggest all of you to get familiarized with these terminologies very well.

So, briefly I will try and explain how the rodent head should be fixed before you begin and that is very very important. The actual rodent microsurgery I will be dealing it with in next lecture. This is a sort of to explain the stereotactic principle you need to sort of understand how the rat will be fixed only then you will sort of understand how the rodent atlas can be used and how those coordinates can be applied to target particular structure. So, one need to know the structure called ear bone spur. So, that is the structure which you can actually see it in the naked eye when you lift the rat in that way.

You know the ear of the rat should be lifted up. So, you can see the bromine protuberance there where a thin layer of skin will be adhering to the bromine spur just behind that is a good point to insert this ear bar into. Otherwise it is a very common problem that you will roughly put into somewhere behind the ear or some in front of the ear or maybe on top of the ear and it slips midway through the surgery. And when it slips your drill slips and it hits a very vital structure like brain stem or cerebellum and the rat bleeds to death. So, it is that vital to ensure that ear bar is pushed in the right place. And to make sure that it is in center one need can one can use the scale that is given here in the ear bars this is a slot for the ear bar and in front and back of that slot you will see a in the back side of the slot actually you will see a graduation like that and there is a 0.

And from how far into how far medially and how far laterally sideward you need to see that and then adjust the similar distance on the opposite side and that will ensure that the head rest in the center or the head rest in the midline of the stereotactic apparatus. So, the middle of the stereotactic apparatus where the nose bar is going to come that should pass through the midline of the rat. So, that orientation is very very important. So, that not only it is rigid your coordinates are also going to be accurate when that fixation happens.

So, that is the ear bar fixation. The next important thing is here is the tooth bar fixation wherein the incisor of the rotator is important. Obviously, these are anesthetized rats and

then you place the incisor teeth of the rat on to the tooth bar, alright. So, once you place that you can give a tug on the skull and then make sure that is the optimal position for your ear bars to go in. For example, this can be as I said this particular you know part of the equipment can be moved front and back based on the screw which is a nut which is given here which can be tightened or loosened and then this movement can allow for the optimal position of the ear bar that comes from the sideways, alright. So, once you fix that there should not be any sideward movement or front and back movement once all these screws are tightened for nose bar and the ear bar.

So, one need to ensure that after that this nose bar is kept on the nose in the nostrils of the rodent. But one should ensure that it is not too tight to allow the air entry. The air entry actually happens there and most of the time you will be keeping an oxygen delivery cannula there through which the air flow will happen. So, one need to make sure that this is not too tight which will compress the nose of the animal. Though the bony canal is bony canal will ensure, but if it is too tight it might can it might even fracture especially if you are dealing with a very small animal like mice or very young mice.

So, it can cause fracture and obstruct the nasal airway. So, that one need to be very careful about. But at the same time one need to ensure that it is rigid enough for not to allow any extra movements that can happen during the surgery. So, this is the this is what I was trying to tell you about the centering of the ah animals head. So, once the nose bar is fixed and the ear bar is fixed it should pass through the midline of the rodent's head.

So, that will be the bregma and that should be the lambda. So, a little bit of that side and this side is fine, but one need to ensure before you start that this midline is in line with the animals midline. So, that is very important. So, as I said that will ensure the rigid fixation and of course, the coordinate as well right. So, once you once we ensure the ah the centring of the head then then only we can ah move forward with the surgical aspects. So, once you make the midline incision and retract the skin sideways then you can see this anatomical landmarks which we just discussed.

So, that is called bregma and that is lambda. So, I will spend some time to make you all understand how these points are formed and what if these points are not in a way it is supposed to be and if there are a sideways because naturally these are natural anatomical structures variations are very common I understand. So, when there are variations in these points one need to ensure that you correct those variations so, that you can use the atlas coordinates here. Unfortunately the image guided a neurosurgery is not yet invoke for animals though there are many labs which are started using image-guided neurosurgery one is that it is very expensive and those imaging are only a reference images which are available for some animals. So, until it becomes the norm one need to

ensure that we are using the reference points very carefully.

So, this is the coronal suture and that is the lambda suture lines. Suture lines are where two bones meet with each other and they fuse when they fuse at this suture line what you can see this squiggly lines will actually disappear, but until then these suture lines which are visible those are coronal and lambda suture and this is the sagittal suture when these two sutures meet here then that forms the bregma point by the sagittal suture meet with the coronal suture. So, that is our reference point from which the target will be chosen for example, this target will be 2 millimeter lateral and 2 millimeter posterior to the bregma that is how the atlas tells you when they say plus 2 millimeter medial-lateral and minus 2 millimeter anteroposterior this is what they mean right. Something to the right will be plus something to the left will be minus something posterior or backward is minus and forward is plus all right.

So, when they say plus 2.3 it is moving in front of the bregma that is 2.3 millimeter in front of bregma or anterior to the bregma when they say minus 2.3 it is 2.3 millimeter posterior to the bregma. So, that is how the bregmas used as the reference point, but before all that once we expose the bregma and lambda what one need to look at is how is these two structures aligned with each other. Only then one can actually ensure that the or coordinates when they say the medial lateral coordinate is in correct position.

For example, this is the surveyed bregma, surveyed bregma is bregma which is visible to the naked eye. For example, in this you can see that the sajjal suture is meeting the coronal suture somewhere there and then this coming back. So, when that happens you need to sort of correct it to the midline because if you take this as a reference point already around a millimeter lateral to the midline and all the coordinates will the coordinate is under the assumption that bregma is in the midline. So, when this naturally occurring bregma is lateral to the midline one need to correct the bregma then you know proceed from there.

So, how do we correct the bregma is this way. You have this arc around you know a smooth arc on the coronal suture and you stop where the lateral edge of the parietal bone beyond that will be the temporal bone there which is this is the sharp edge where the temporal muscle gets attached. And when you start doing the experiment you will see that this is a very prominent ridge in the lateral sideward aspect whereas, here roughly would be the lateral edge. So, when you join those edges in a smooth curve like that the summit of the curve will be the midline. So, you can use the exact center point of this arc to draw the midline and then say that that is the corrected bregma. So, the midline is passed through the center point of the arc forms the corrected bregma alright.

So, then that will be used as your reference or if you have a digital display you need to make clear it and make it 0 when it reaches this rather than this point. Few millimeter this way and that way makes a lot of difference when you are using the deeply situated targets. So, that is the reason why one should ensure that your bregma is corrected alright. Similarly lambda if your reference point is lambda for example, if you are targeting any structure in the cerebellum you probably would use lambda as a reference point and you need to ensure that midline even for the midline is very important when you go posterior so posteriorly you will be drawing the line in the midline starting from the bregma. But if you are trying to meet the surveyed lambda it is it is sort of already you know move to a side and that is not the true anatomical midline of the rodent alright.

This is very important for a simple reason that there is a structure vascular structure called sagittal sinus. If there is a structure that you are going to target which is very close to the midline you need to know how to angulate it and then target which I will be talking about in detail. So, when we do that this midline orientation is very very vital. If there is a structure which is you are just trying to sort of ah move a bit lateral and posterior to bregma it does not really make much of a difference from the lambda. But if it is a deepest deeply situated structure is only very few millimeter away from the midline then this midline orientation is very important and you need to sort of correct the medial-lateral coordinate starting from the reference point.

So, what we ah earlier ah commented about the corrective bregma similar thing also stands for lambda. Here what you are seeing is the green ah structure that is the surveyed lambda that is after you expose this is what you are seeing alright. You are seeing the lambda here because if you see that closely that suture line is meeting the lambda suture left lateral to the midline not exactly ah you know in the midline is is to the left of the midline. So, then you can bring it back to the midline by using the same principle which I explained that you take the corrected bregma and then pass a central line draw a straight line and that to ensure that lambda is corrected to the center back into the ah central plane that forms the corrected lambda. So, then you then on then on then only that you will be able to use the coordinate and proceed further after correcting the lambda and the bregma.

So, another very ah vital ah you know the vital ah point that one need to keep in mind before proceeding further after you expose the skull is that you need to make sure the skull is flat in orientation. They should not be any ah you know there is any inclination when it comes to bregma and lambda. This flat orientation of the skull is pretty important before you target the deeper structure and how do you ensure that is that you bring the ah needle or a marker on to the bregma and you notice a coordinate. You will write down the z coordinate or the display that you make it you clear it the digital display

you clear it and make it 0 or if you do not have a digital display you note down the z coordinate. Once you move into the bregma you need to see how much you have moved midolateral, how much you have moved ah anteroposterior, how much upward and downward movement you have done based on that you notice the ah scale from the ah arms and then write down the coordinate for the bregma alright.

Once you write down the coordinate then you will move your adapter to the lambda point and again you touch the lambda point using the needle that you have fixed onto the adapter. Then again you write down the coordinates the difference should be less than 0.1 mm alright. If it is anything more than 0.1 mm this is what is going to happen. If the lambda is at higher position than your bregma then this is how the target is going to shift because you are going to use the ah entry point from the skull and you will target the structure, but you will hit the target which is posterior or back of the intended structure not exactly the structure that you want to really hit. And for example, if this ah targets a structure is only around let us say 2 mm in size 2 millimeter in size the shift is already 2 millimeter you will entirely miss the lesion and not only you will be a targeting and creating lesions and you will be seeing entirely different behavioural change in your experiment. And unfortunately you will blame it on various other problems like your equipment failure or rather the behavioral training issues. So, you will miss the ah problem entirely if you are not familiar with this flat skull orientation. So, before proceeding you need to correct the bregma, correct the lambda and then correct the ah flat skull ah orientation by elevating the nose bar alright.

This is where that adapter with the adjustment tool comes into rescue where you can you know raise the ah tooth bar and nose bar on top until you correct the bregma and lambda into a flat orientation as I said it should be less than 0.1 mm. So, in this example the bregma is lower down and this is where I said the tooth bar and nose bar can be adjusted to raise it to make the skull flat. And in this example one can see the bregma is too high and lambda is low in which case the intended structure is you know posterior or sorry anterior to the intended target alright. So, this is the structure which should have been targeted, but you will be hitting the structure which is in front of it.

So, this is basically to explain that how your trajectory is going to change based on the relation between the bregma and lambda. So, this is ah very vital when you are targeting a structure. For example, if you are doing some surgery and implanting something on the surface this does not really make much of a difference because you are making anyway craniotomy you are exposing the brain anyway. So, it does not really make much of a difference, but if it is the deeper target to create any lesion or inject any drug into the structure this is of very vital importance.

So, make sure your z coordinate for bregma and lambda is less than 0.1 mm difference alright if it is more corrected as I just explained alright and for that you need to correct the bregma and lambda as I explained earlier alright. So, another very important thing one need to remember is that when you go away from the midline there is an arc that that is going to come in the skull is not really flat as you move away from the midline it is in the arcs structure and you are losing the depth of around 0.9 mm if it is the lateral edge lateral most edge of it. So, though the atlas will say the z coordinate is 3 mm you need to account for this loss of around 1 mm close to 1 mm when you are when you are using the lateral most entry point alright. If it is like for example, 4 mm lateral from the midline that is quite far away from the midline and you will be sort of losing around 1 mm before you start eating the brain or the difference is sort of 1 mm and you will be eating the brain earlier in the lateral edge because brain will be almost here and you will have some depth of around 1 mm when you reaches close to the midline.

So, that sort of difference also need to be kept in mind before we before you use the z coordinate in the lateralmost entry point. So, before I proceed I would want to introduce this particular atlas which is sort of a bible for all Rodent stereotactic neurosurgery which is Paxinos and Watson atlas. Very important to familiarize with this atlas because if suppose you do not really get a literature which can give you the coordinates and I would say even if you have literature coordinate based try and understand the structure that is the target in question or your target of interest you need to understand using this atlas even if you have a coordinate because you need to understand what structure comes in front of your coordinate what structure comes posterior to coordinate because if suppose you see a behavior which is little deviated from the expected behavior then one can easily understand if you can understand the anatomical relation around the intended structure. It is very vital to understand the atlas and how the atlas is prepared and what are the reference plane they have used how to interpret the pages in the atlas to identify the structure that you would want how do you really gather the knowledge about coordinates to target in the rat that you are going to use for experiments.

So, they have used the vista rat typically male vista they have used around 290 grams weighing 290 grams. So, that is the size of the rat they have used and what is been shown here is the interoral line and the reference points again bregma and lambda and that is the incisor bar that comes here and that is also very important that some target which are deeper you need to sort of give inclination before start targeting it. So, you need to understand how much is the height that can be achieved from the incisor bar there all right. So, apart from that I would sort of want to introduce the atlas and the coordinates as to how to calculate various and what are these you know grid lines actually refers to. So, everybody is familiar now with the sagittal section of the brain rats brain and that is the anterior part of front or posterior or back portion wherein that is

cerebellum that is cerebellisocortin that is the olfactory bulb there. Let us say if you want to target a particular structure here anyway I will be covering an example there, but then you need to know what is the depth of it to achieve and how far you need to back move back from the bregma there all right and then obviously, you want to know how many slices you need to go lateral you know sidewards from here.

So, this will sagittal orientation will give you the depth of the structure and how far back from the bregma, but you need to choose a particular slice which will give you the maximum dimension of the nucleus in question that is very important. Let us say there is a pediculotegmental nucleus or subthalamic nucleus let us say that measures around 1 centimeter. So, you need to choose a slice which has highest dimension all right because as you move sagitely and if the orientation of that nucleus is in this way and the sagittal section has gone that way and if you choose the slice which is covering the lateral most that is a very small area and it is very easy to miss targeting that if you choose a slice and take the coordinate from that slice. So, one need to be very cognizant about the fact that choose a slice which is gone through the maximum width or height or length of it, but you need to get a big room for targeting that particular structure that is very important. Then only after selecting a particular slice then only you should look at the coordinates and then write down how many millimeters behind and how many millimeters deeper.

So, here everything is what shown here is on 1 millimeter apart the each grid line is 1 millimeter and here you will see a notation called lateral 0.4 millimeter that is very important to understand because you this is what is going to give you a coordinate for side words. So, for example, you got the depth here let us say if I am using this, this will be 7 millimeter deep alright and 15 millimeter posterior to the bregma. So, that is Ap will be minus 15 and the z will be 7 mm and you need another coordinate which is medial lateral that would be lateral 0.4 alright.

If it is light right lateral sides depends on which limb are you trying to study or which side of the rodent body you are going to study that decides the side whether it is right side or left side. If it is right side will be plus 0.4 mm. So, these will be your coordinates that you are going to use it on the stereotactic setup. You will use these coordinates just through the vernier scale you will you will adjust for a 15 mm in the anteroposterior axis 7 mm for you know dorsal ventral axis and medial laterally you will use 0.4.

So, that is how you arrive at a coordinate using the atlas. First of all choose the particular slice which give you a maximum dimension. Once you chosen the slice in this sagittal that is how you calculate the coordinate based on the ah location of your ah target. So, that was the sagittal section. In a coronal section again it is very important to correlate and see that your chosen 0.4 How big is the structure in when you see a 0.4 millimeter away from it all right.

So, if this is 1 mm then you will see each of these will be 2 divisions that would be 0.2, 0.4, 0.6, 0.8 and 1 mm. So, each of these grid lines is 1 millimeter as I said and the entire breadth of the atlas the given is the 16 mm ah you know the width of the ah each slice and what ah I want your attention is for bregma and interaural distance that is very important to look at again. So, this is the orientation of this particular slice and if you look at here carefully you will understand what these numbers are.

Bregma is point ah minus 1.08 millimeter. So, if you see that is ah bregma point there where that is 0 and then it is 1.8 mm posterior or back from the bregma that is why it is minus 1.8 and this line in the atlas actually tell you that is where they have taken the slice from. Then there is something known as interaural ah line as well. So, there there are 2 ways of looking at the interaural line. If you all remember basically that line mean interaural line is that when the rat is there you put the ear bar the line passing through the ear bar will be the interaural line and the line of fixation.

So, you can look at it 2 ways that for an axial slice it will be it will give you the ah dorsal ventral orientation of the slice that is going through. For a coronal slice like this it will be saying how many if for example, if that is the ah interaural line which goes from ah sideways you will see how many slices and the how many ah millimeter anterior to it and the bregma is giving ah how many ah millimeter behind ah or posterior to bregma.

In this example you will see that interaural distance is 7.9 millimeter that is 7.9 millimeter in front of the interaural line. So, that is about the coronal orientation. Next is again axial orientation. Here again you will see the bregma and the for the interaural line. So, bregma basically is going to ah tell us that the depth from the bregma you know this is the slices are going in this way.

So, the depth of the slice is minus 5.8 ah 5.8 mm and interaural is 4.18 mm. So, you can see that from the interaural ah line they moved 4.18 sorry mm above to take the axial section. Whereas, here the 0 again it refers to bregma no doubt and if you are choosing a coordinate of the if you choosing a target there then you know how how many millimeters posted to bregma and how high from the interaural line. So, these are the different ah planes of orientation where once you chosen the structure you check it again that you chosen a correct slice or not. If suppose the orientation for example, that is the ah I would say this subthermic nucleus there and the various ah nuclei here which is left post occipital and then you have this subthermic nucleus there.

You will see various nuclei in a coronal orientation the breadth is more. So, you can choose this to target and then move from there rather than using a sagittal orientation

where the nuclei ah depth is pretty less. And if you see the ah M 1 which is the primary motor cortex M 2 will be the secondary motor cortex and you see the globus pallidus which is pretty large in the coronal orientation. So, you can choose the lateral distance there and then the vertical z z distance then you will get the ah antroposterior axis of bregma which is an AP coordinate which is of minus 1.082 target this particular structure.

So, that is the brief idea about ah how do we choose a target and how to calculate the various coordinates alright. So, then we will discuss about the few examples I have shown as to how a particular target is chosen. So, this is one such example where the substantial Niagara Pars reticulata has been chosen as a target alright. So, that target if you see as I said medio lateral the final coordinate is medio lateral plus 2 that is to the right the plus 2 they have chosen the coordinate you need to move from the midline laterally 2 millimeter and AP is 5.8 which is minus 5.8 as I said this bregma is going to give you the AP ah this is in the atlas this is exactly how the atlas will give you the ah images for various targets.

And as I mentioned earlier these atlas are prepared by taking serial sections you know at 1 millimeter section and then taken photographed and then used as the atlas. So, all these structures are going to be the structure how it is this is how it is going to be in the rat that you are going to do the experiment on. So, when they say it is minus 5.8 mm there will be some amount of a variation, but by and large if the structure is that big that variation is sort of acceptable alright.

So, minus 5.8 becomes your anteroposterior ah distance that is minus 5.8 millimeter posterior to the bregma that is where the slice is taken and that is where your ah structure is largest in the dimension. And then I guess the dorsal-ventral coordinate is not seen in the screen, dorsal ventral is minus 7.82 whereas, if you see this x mark which is center of the target is actually 8.2 mm alright.

But if you want to leave the drive cannula above the ah target so that the final ah needle or micro-inflation needle can hit the target. So, if you want to use your drive cannula you can leave the room of around 1 mm and then leave it just above the ah target not exactly going inside and damaging it because the dimension of the drive cannula is going to be bigger than the actual needle which is going to go in alright. So, that is how accurate it is and that is how we can sort of titrate and choose whatever distance that we need to calculate. So, once you chosen a target then you choose the center of the target then you look at the various coordinate as I just explained. And but there is a difficulty will come when you choose the structure which is dead in the midline.

For example, this is the ah ventral diagonal band of broca alright this structure actually comes in the midline. But the problem is if you remember the anatomy that we discussed there is this big saggital ah structure called saggital sinus right in the midline is a very vital ah vein which takes away the ah blood from the brain to the heart. So, the deoxygenated blood goes through the venous system and this is the very important venous channel which is right in the midline alright. So, there is no coordinate that can be chosen as an entry point right through the center which will kill the rat instantaneously. So, one has to avoid the entry point in the midline you need to go sort of laterally and then enter and hit this target.

So, obviously, you will be taking that trajectory. The problem when you move sidewards is that the distance to reach that particular target is going to change alright. So, when you use the atlas this is the difficulty that you are going to face. So, how do we sort of ah modify that and work around that point is by using this particular technique sorry yeah. So, here you can see that there is this arm that is available to swing in an angular the entire arm can be lifted up and you know it can be swing in the arc which is given in the base of that particular arm.

So, this adapter holder can be moved up and down and can be tilted to various degrees that are given in this ah graduated arm base. So, here in this example all we need to do is to move around 1.3 to 2 mm to avoid the midline venous sinus. So, here in this example they have moved 10 degree alright they moved 10 degree in this ah graduation and then attempted to target this particular structure. So, when you move around 10 degree when you swing around 10 degree what will be the distance that needs to be calculated that you will get it using this particular formula.

All of us are familiar that cosine alpha for the adjacent divided by hypotenuse. So, when we calculate that we know the dorsal ventral coordinate for according to the atlas was 7.4, but then that goes through the midline and will hit the venous sinus that is this.

The amount of angulation that you have given using this arm is 10 degree. So, cos 10 is 0.9848. So, using this formula we will get a new trajectory distance of 7.51. From 7.4 we have chosen 7.51 as a new distance which is the depth of the ah trajectory to hit the same target which is in the midline by avoiding the venous sinus. So, that is the ah end of the session today. So, we have covered most of the stereotactic principles and then tried to troubleshoot and tried to know how to target ah particular structure, how do we choose ah structure from the atlas and then use the coordinate from the atlas. So, it would be very useful if you all can shift from the literature-based coordinates to the atlas-based coordinates, especially when the structure or the target that you want to study is very new and obviously, the new coordinates from the literature are going to vary. So,

use the atlas which has the similar species what ah to similar to your experiments and then calculate the coordinates from the atlas and apply it on to the stereotactic setup. So, we will be ah I will be taking you through the various surgical steps though it did sound pretty ah easy to do all this stuff, I would suggest that we are planning to have ah animal lab workshop as well where these has to be you know tried hands-on.

If in your lab I am sure there will be someone who is doing it, but unless you assist them and you know try yourself ah you know doing it, it is very difficult. There is a significant learning curve that one has to go through before starting using these stereotactic setup, but nevertheless it is easy to crack it once we understand the principles and once you know how to troubleshoot all these problems. So, the whole idea of this is that so that the mortality and morbidity for your animal subjects will come down and the number of rats that are used also will come down. So, in the next session we will deal with the stereotactic surgery and various other neurosurgical steps in detail. Thank you.