Cell Culture Technologies
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Lecture – 40
Advance Cell Culture Modules – V

Welcome back to the lecture series in cell culture. So, we are in with of fifth lecture of 8th week, lecture 5, week 8; so W 8, L 5.

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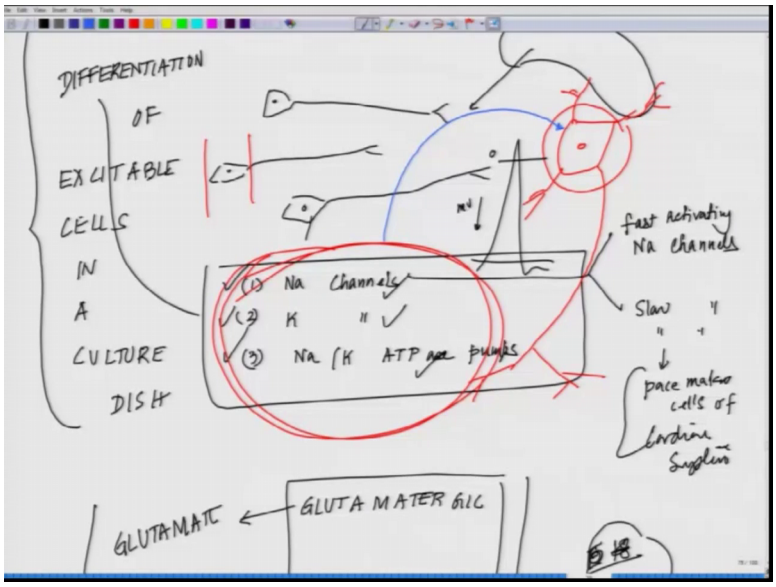

So, in the previous class, we talked about 4 classes, we talked about how to evaluate the force generated by the muscle using micro electromechanical systems, how to make a neuromuscular junction outside in an in vitro setup. Now we will talk about another interesting challenge which is faced by most of the excitable cells and I am pretty sure, this is faced by people who work on insulin secretion cells and all those kind of other cell types is the electrical excitability when we remove a cell or we remove a neuron from the nervous system we are dividing it in a culture dish without the Glial cells and other supporting cell types.

And what happens is that most of these cells which we put on the culture dish, say for example, you take out these hippocampal neurons. So, from the hippocampal you take out the neurons. So, what you have is nice pyramidal neurons growing out on the culture dish something like this, now the problem with such culture is are these neurons shooting

action potentials or not if they are not shooting an action potential, then they are really of no use because a neuron which is not electrically active is not really differentiated the way it should because electrically active neuron means it should have an expression of the necessary sodium channels potassium channels sodium potassium ATP-ASE pumps and within sodium channel, it should have an expression of mostly in the central our system of course, fast activating sodium channels and some of them of course, are slow activating which is in the pacemaker cells and likewise sodium channels this is exclusively on pacemaker cells of cardiac system.

So, these all things falls under your differentiation in other word you can term it as electrical differentiation of neurons in a culture or any other excitable cell as electrical differentiation of excitable cells in a culture dish. So, in this fragment, I will take 3 situations; how this is being achieved. One of the ways where you are growing pure hippocampal neuron from fetal day 18 rats one of the things, what many people does is that they while plating the cells, they add very trace amount of around 25 micromolar or even much less maybe 20 micro molar or maybe 10 micro molar of glutamate glutamate is an excitatory neurotransmitters and most of the nervous system or the hippocampal neurons.

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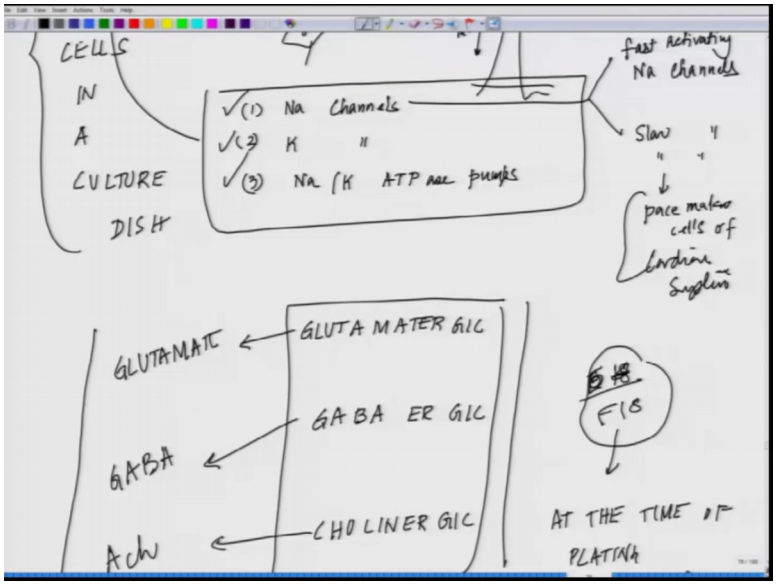

So, if you classify the hippocampal neuron they, they falls under 2 types, either it will be glutamatergic or it will be gabaergic or a small population which is cholinergic,

especially this is the equation at E 18 or sorry F 18 should call it embryonic, it is a fetal stage.

So, now, glutamatergic neurons are the one which secretes glutamate as a neurotransmitter gabaergic as the one which are secreting Gaba is the neurotransmitter cholinergic are the ones which are secreting acetylcholine are the neurotransmitters. So, on F 18 culture at the time of plating one has one adds a very very very very trace amount 20 to 25 micromolar of glutamate, the reason is this glutamate helps in the expression of or in the differentiation of some of these channels and further bring and x brings an excitability by binding to the glutamate receptor on their surface.

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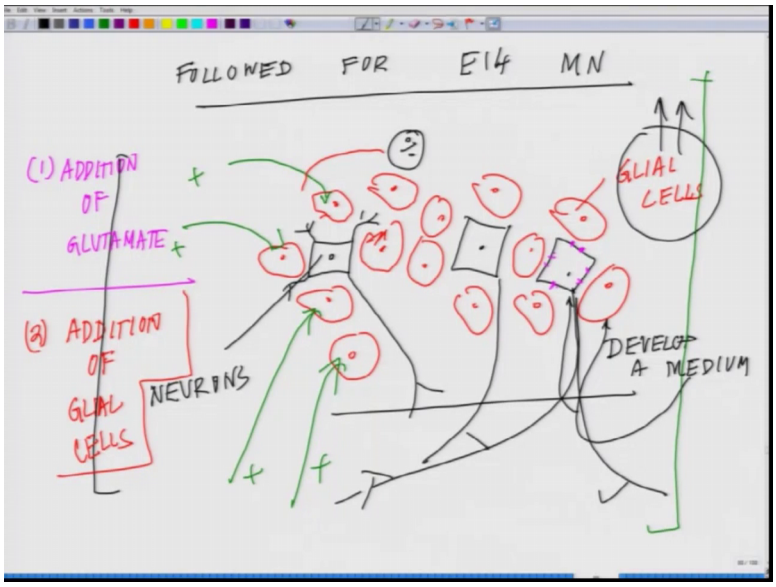

So, this is one technique by which and this is the same technique which is followed for E 14 motor neurons a very similar technique which is being followed for E 14 motor neuron to make them electrically excitable you had very very trace amount. So, you have to be very cautious with the glutamate because a little bit of a higher glutamate could lead to toxicity within their cells in nature. Now we might wonder; how in our body that toxicity does not happen of course, it does happen in people who suffers from hyper excitable disorders like epilepsy, the reason being our neurons in the system are surrounded by a wide number of glial cells in and around and these glial cells have this enormous ability and they are much larger in number.

So, these glial cells pull away those excitatory neurotransmitters from the system very fast. So, in other word those glial cell acts as a sponge. So, here you have the glial cells here you have the neurons. So, now, if you want to do a very pure culture that you do not want any glial cells to be present, there learning have to be very cautious that you are not adding enough glutamate to damage the cells and yet you are not adding. So, less that the cells do not get activated. So, this is one paradigm which I wanted to discuss. The next paradigm is this where I will give you some papers you will see addition of glial cell in a culture dish increases the electrical excitability of the cells again a very interesting study where on a micro electrode array, these cells were grown and the neuronal cells on top on that you add the glial cells or you have the glial cells on that you add the neuronal hippocampal neurons.

And then you see how that gets affected its very interesting to note the addition of glial cell helps in the expression of the different kind of ion channels which are present in the neurons and the series of other things which are involved with it. So, first paradigm is I told you about addition of glutamate step, this is one way you can do it, the second way is addition of glial cells the addition of glial cells brings a different set of problems, the different set of problems is these glial cells are I told you in the previously these are dividing cells. So, now, you have to add them and. Secondly, you have to have develop a medium which will prevent the proliferation of these glial cells too much and of course, we will not influence the neurons too much.

So, you have to have a balancing act in terms of the medium what you are developing which will essentially ensure that neither your glial cell number is going. So, up that it is affecting the connectivity between the neurons and it consumes. So, much nutrients that the neuron gets deprived of nutrient nutrients second you have to ensure that the neuron can fully grow and make connectivity on a bed of glia. So, these are some of the very interesting problems what people are addressing for last almost more than twenty years or last 2 and a half decades that how to develop these kind of in vitro setups which can be used for some amazing experiments what we can think of now in the same line the story becomes even much more complex.

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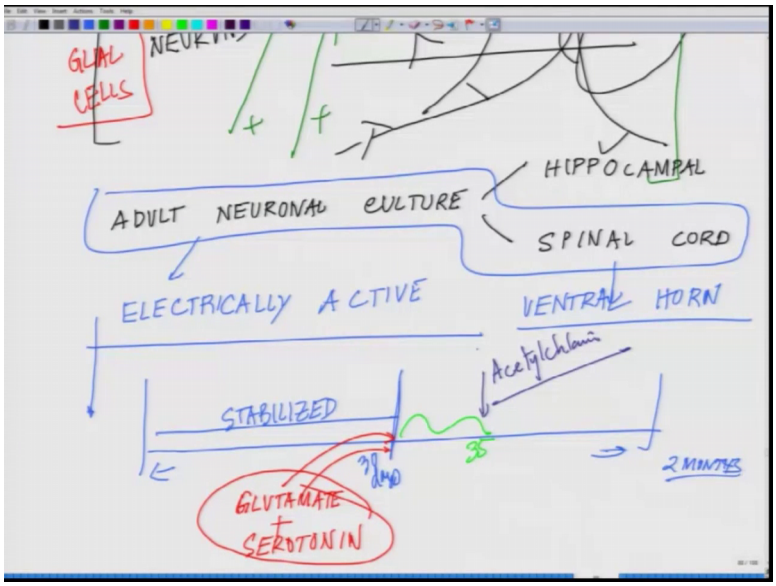

When we talk about adult neuronal culture one of the major challenge in adult neuronal culture especially whether it is hippocampal or whether it is spinal cord is how these neurons becomes electrically active.

Because you have to realize the clock by virtue of which these cells develop all these ion channels this happened at the very early phase of the development when we are growing in a mother's womb most of the neurons acquire disability and we know that neurons in the body at least do not divide in a culture dish they do divide, but in the body as of now we are not very sure, when there are contradictory evidences, but much of the hippocampal neurons major ones or motor neurons they do not divide. So, once these are formed these are formed you know there is only turnover now say for example, you take an adult neuron what you get in a dish is something like this and this again sends out the process which is an adult neuron its extremely challenging kind of culture, but it does.

So, they send out the processes and they form on the connectivity and everything, but now while doing it, it has to realize it has to follow the time, what it has experienced at the very onset of development of that now in order to do. So, you have to create a menu where you can achieve this and this is not something a trivial problem. This is a problem which bogged at least me in one of the in vitro systems what we were developing where we wanted to have adult spinal cord culture which is electrically active means the cells exhibit electrical activity, how to achieve it one of the ways at least, what you have

documented in this paper will be there for you to read through we allowed the culture to grow for. So, we first of all established adult spinal cord culture adult ventral horn of course, where there is a huge population of motor neuron surviving motor neurons apart from of course, there are some glial cells here and there are inter neurons.

So, we allow these cells to survive in a culture for 2 months first of all we set it up like that at least for 2 months; 60 days, they grow and after first 30 days in the culture well the culture is all well stabilized we add 2 neurotransmitter and this once you see through the paper, you will realize it was after a lot of trial and error we had to go through, we add glutamate plus serotonin, these are some of the neurotransmitters which helps in the development of the nervous system and then after another 5 days, we allow them to you know kind of you know after 5 days which is on a 35th day, we add another neurotransmitter which is acetylcholine.

So, in other word what we are trying to do was we were temporally conditioning the neurons.

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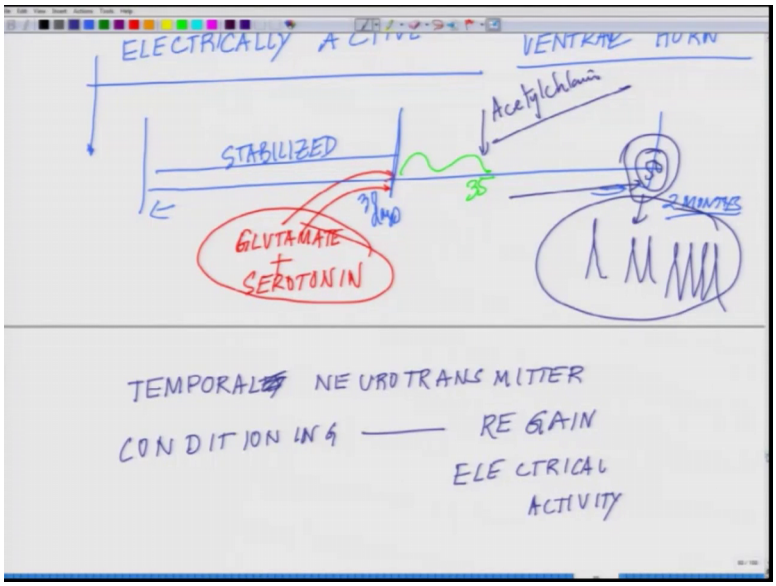

In a culture dish temporarily or they can call it temporal neuro transmitter conditioning your conditioning the neurons over a period of time in order to regain electrical activity most of the cells were not electrically reactive, so, coming back here. So, then you allowed them to grow for another fifteen days day fifty somewhere around this forty to

fifty he started to evaluate their electrical activity and what you observe is most of the neurons at this stage are shooting multiple double single action potentials.

So, this was one of the discovery which add a new set of flight into the cultural paradigm that how ion vitro culture systems can come more close to reality, where we can achieve what we can achieve with embryonic cell type, but what he can achieve in embryonic cell type cannot be a real life model for age related disorder. So, for age related systems you needed something which is age related matching systems. So, when you grow adult cells adult neurons adult excitable cells these are some of the challenges what you come across and you have to always device innovative ways to deal with these kind of situations you really do not know because these are the models or the paradigms which has not been followed.

So, when you are the first one it becomes even more challenging to realize that you know you have to think out of the box and this is one idea this neurotransmitter conditioning which we have to think way out of the box and because the problem is that adult cells when they are regenerating when you are culturing what isolating the tissue you are pretty much breaking all the processes, it is a single entity coming out the cell body and it has to you know resend all its processes you have to realize that these cells are very fragile very very fragile and it is not easy to grow them it took several years for us to standardize some of these techniques as a matter of fact almost more than a half a decade it took 5 to 7 years, it took us ready to get a hang on the system. So, in such systems, you cannot rampantly use any kind of neurotransmitters. So, easily because they are so fragile, they are they can get excited hyper excitable or they can; they may get; they may get damaged or injured very easily.

So, that is why well we are setting up this paradigm, we said let them grow for some time you know significant period of time. So, we allow them to grow for almost 3-4 weeks before we started tinkering with the system. So, you realize growing neurons that long is also not a trivia it takes enormous effort to make these things happen, but the whole field of in vitro biology or in vitro cell culture is a slow moving field and there are a lot of emerging technologies which are coming up mostly from the engineering background which will change the face of this whole area in years to come. So, what I will do after this is I will give you 2 more. So, this is pretty much finishes the course.

I will give all the papers what I have promised, they will be uploaded and apart from it, I will give you 2 more small modules of micro channel technology which will help you to realize some of the other application of the MEMS fabrication which are being used and apart from it, you will have skew papers on micro electrode arrays and if it is in cell culture and achieving electrical excitability.

Thanks a lot thanks for your patience listening.