Module:
Biological Foundations of Mental Health

Week 2
Building blocks of the brain

Topic 1
Neuron-glial interactions and mental health – part 2 of 2

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Lecture transcript

Slide 2

In part one, we've seen how astrocytes, arguably, play an active information processing role in the CNS further to their supporting homeostatic role. Therefore, it is logical to conclude that astrocytic dysfunction may well contribute to the development of mental health disorders.

Furthermore, we could argue that the lack of universally effective pharmacological treatments, or other form of treatment, for mental health disorder is due to the currently predominant 'neurocentric' approach to the study of human behaviour or mental illness. This neurocentric approach has not allowed us to gain a full understanding of the mental illness. Therefore, we could also argue that an alternative, gliocentric view, may lead to a better understanding of the basics of mental health disorders, leading to more effective therapeutic strategies.

Slide 3

Before moving on to discuss the evidence for a contributing role of astrocytes to mental health disorders, we should attempt to define what these disorders are. According to the current edition of the Diagnostic and Statistical Manual of Mental Disorders, DSM-5, which is used by clinicians and researchers to diagnose and classify mental disorders: 'A mental disorder is a syndrome characterised by clinically significant disturbance in an individual's cognition, emotion, regulation, or behaviour that reflects a dysfunction in the psychological, biological, or developmental processes underlying mental functioning.

Mental disorders are usually associated with significant distress in social, occupational, or other important activities. Examples of mental disorders include depression, bipolar disorder, schizophrenia, autism. One significant common feature of these disorders is that, in general, their aetiology and pathophysiology are not fully understood. However, we do think that a variety of genetic and environmental factors are responsible for the onset.'

Slide 4

Why do we still know so little about the potential role of astrocytes in psychiatric disorder? It is very difficult to study these in humans. Since the alterations may be subtle, people do not usually die of psychiatric disorders and at the time of death, past history of the disorder may be ignored, other illnesses may mask changes caused by psychiatric problem, alteration caused by pharmacotherapy for the disorder may be indistinguishable from changes caused by the disorder itself.

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Slide 5

The main line of evidence that are available to formulate hypotheses on the environment of astrocytes in mental disorder derives from a range of studies in different part of system.

First type of study are human study, which are mainly postmortem. The second type of studies are animal studies, including use of genetically modified animals. The third line of evidence is in vitro studies, including astrocyte cultures, brain slices. For example, pharmacological studies on the effect of currently used therapies for a variety of neuropsychiatric disorders on glial cells, in vitro.

We shall now examine some of the evidence currently available for an involvement of astrocytes in mental illness, and we shall particularly focus on major depressive disorder. However, mention will be made elsewhere of evidence available for schizophrenia.

Slide 6

Before discussing the evidence for a role of astrocytes in depression. What is depression? It is a common mental disorder that causes people to experience depressed mood. It's characterised by loss of interest or pleasure, called anhedonia, feelings of guilt or low self-worth, disturbed sleep, which can present as insomnia or excessive sleep, low energy, poor concentration.

What are the neuropsychological processes underlying a depressed state? There are a number of theories on the neurophysiological processes underlying a depressed state, obviously, focused on neuronal dysfunction, according to the neurocentric view. And currently the dominant theory is the so-called monoamine hypothesis, which states that depression is the result of under activity of monoamine neurotransmitters, especially serotonin. Indeed, most antidepressants aim at increasing the level of available serotonin or monoamine neurotransmitters in general.

It is now also thought that dysfunction of the hypothalamic-pituitary-adrenal, HPA, axis, a system which is involved in the response to stress, may be implicated in the pathophysiology of depression. Circadian rhythm abnormalities leading to disruption of sleep patterns have long been thought to play a role in mood disorders, including depression. And finally, neurodegenerative and inflammatory alteration may also be contributing factors, particularly in late onset depression.

Current pharmacological treatment has variable efficacy, but usually between 30 per cent and 50 per cent of sufferers will not respond to a specific antidepressant medication, and about 50 per cent of sufferers are poor responders to pharmacological treatment in general.

Slide 7

So what evidence do we have that astrocytes may play a role in depression? There are actually several lines of evidence that suggests that astrocytes may play a role in depression and they are derived from studies in animal model, studies on postmortem human tissue, and finally studies on astrocytes in culture. And we're now going to examine some example of each of these type of studies.

Slide 8

Let's start with some example of studies in animal models. In animal models of depression, an astrocyte pathology is present. Treatments that revert the astrocyte pathology also revert the behavioural symptoms of depression in these models.

Slide 9

Chronic unpredictable stress is used as an animal model of depression. In this model, animals are

subjected for a number of days-- 35 days in this case --to exactly the same sequence of 12 stressors, two per day. Example of stressors include cage rotation, light on, light off, cold stress, isolation, crowding, cold swim stress, et cetera.

In order to assess the impact of chronic stress on astrocytes, the authors of this study measure the level of messenger RNA for a specific marker of astrocytes, which is called glial fibrillary associated protein, or GFAP, by a technique called in situ hybridisation.

The histogram in A gives the percentage of messenger RNA for GFAP - it's percentage of control - in animals kept in home cages, so not exposed to any stress, that are indicated here as CTR, and animals exposed to chronic unpredictable stress.

The open bars indicate animals treated with saline, and we can see that there is a significant decrease in the level of GFAP messenger RNA in animals exposed to chronic unpredictable stress. This effect of stress can be reversed by injecting the animal with a glutamate modulating drug, Riluzole.

At the bottom are representative autograph of the effect of chronic unpredictable stress on GFAP messenger RNA expression compared to controls.

Slide 10

The authors of this study then employed a test, which is supposed to measure anhedonia in mice. As mentioned before, anhedonia's considered a symptom of depression. Rodents, some of mice, and rats are born with an interest in sweet foods or solution. Therefore, if given a choice between a water bottle and a bottle containing a sucrose solution, mice should preferentially drink from the sucrose solution containing bottle. Reduced preference for sweet solution, in the sucrose preference test, therefore, represents anhedonia, and chronic antidepressant can revert these reduced preference.

Slide 11

Day 15 of exposure to chronic unpredictable stress, stressed animals showed a significant decrease in sucrose preference when compared to home cage control, CTR. Disease presented in the panel in A, where controlled animals are approximately three times more likely to drink from the sucrose-containing bottle than from the water bottle, and this preference is reduced in stressed animal.

The decreasing sucrose preference was even more significantly decreased in animal exposed for 35 days to chronic stress, and this is presented in the two open columns. This decrease was reversed by chronic Riluzole treatment in parallel with the decreasing glial pathology, which was observed in the previous slides. And these are the two columns in black, where both the control animals and distressed animal presented a preference to drink the sucrose-containing water.

Why does Riluzole reverse glial pathology and depressive behaviour? It is proposed that Riluzole, boosting glutamate uptake by astrocyte, would also boost glutamine production by astrocyte, and this can be therapeutic in the context of depression.

Support from these hypotheses comes from studies which show that patients with major depressive disorder exhibit reduced cortical levels of the neurotransmitter GABA. And this is similar to rats, which undergo chronic unpredictable stress. Normalising GABA levels in these individuals correlates with that clinical improvement. GABA synthesis in neurons requires glutamine, which is produced by the astrocytes. Thus, it appears that the dysregulation of astrocytic support of the GABAergic transmission contributes to the pathophysiology of major depressive disorder, and this process may provide normal therapeutic targets for the treatment of this debilitating state.

Slide 12

We shall now move to some example of studies on human postmortem material.

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Slide 13

In these studies by Torres-Platas et al., they measured the level of messenger RNA and protein for the astrocytic specific marker GFAP in various brain areas of postmortem material obtained from depressed suicides.

The authors looked at areas not involved in mood control, such as the primary visual cortex, on the left, and the cerebellum, and areas known to be affected in mood disorders, such as the mediodorsal thalamus and the coded nucleus, on the right.

Both GFAP messenger RNA and protein levels were found to be similar between control and suicide in the visual cortex and the cerebellum. However, both the messenger RNA and protein levels for GFAP were significantly down-regulated in suicide in the mediodorsal thalamus and the coded nucleus samples, indicating the presence of an astrocytic pathology, specifically in areas known to be involved in mood regulation.

Slide 14

We are now going to look at some examples of studies conducted on cultured astrocytes. In these studies, it was shown that various type of pharmacological and no pharmacological treatment for depression can act directly on astrocytes.

Slide 15

In this first study, the authors looked at the effect of a commonly used antidepressant, Fluoxetine, better known as Prozac, on cultured astrocyte.

Prozac is one of the most famous and used antidepressant medication. Its mechanics of action is supposed to be the inhibition of serotonin reuptake in the brain. This would increase the amount of serotonin present and, consequently, serotonergic neurotransmission. However, in the experiment presented here, the authors showed that Prozac can also act directly on astrocytes, and this is shown in the panel at the top. In black are control astrocytes and in light grey, Fluoxetine-treated astrocytes.

As it can be seen, the production of a number of trophic factors is increased in astrocytes treated with Fluoxetine.

The production of BDNF, VEGF, and VGF are all increased in Fluoxetine-treated astrocytes.

Interestingly, this effect is totally independent of serotonin, since treating the cultured astrocytes with serotonin does not induce trophic factors synthesis, and this is presented in the panel at bottom.

In the panel on the right, we can see that serotonin treatment of the same cultured astrocyte had no effect on the synthesis of neurotrophic grow factors.

It is interesting to note here that the full therapeutic effect of Prozac may be delayed until four to six week of treatment. This has proved difficult to explain if Prozac acts via increasing serotonin level at the synapse, because the effect should be almost immediate. However, if the main effect of Prozac is on astrocytes via induction of the production of trophic factors, the therapeutic delay may be easier to explain, since it would take some time for the increasing trophic factor to lead to increasing uptake plasticity, neurogenesis, and restoration of damaged neuronal network.

The evidence presented, so far, suggests the presence of a glial pathology, probably astrocytic atrophy in depression, which correlates with the presence of depressive symptoms in humans and animal models.

The studies that I've shown on astrocytes in culture, treated with antidepressant, also suggest that

potentially the therapeutic effect may be due to action on glial cell. However, none of the studies discussed so far, can confirm a causal link between astrocyte pathology and depression, or clearly suggest what the underlying mechanism might be.

This particular issue was addressed in further studies, utilising again postmortem material and animal models, and we are going to examine some of those.

Slide 16

We saw earlier how astrocyte can be connected in networks, which are not fixed, but can be modulated. We also mentioned how these networks can play a role in integrating neuronal activity. Is it possible that disruption of astrocytic networks may play a role in depression?

Let's look at some evidence from postmortem human material and animal models of depression.

Slide 17

Some evidence for postmortem material suggest that disruption of astrocytic networks may indeed play a role in depression.

As we mentioned in part 1, astrocytes are connected in networks via gap junction, which are composed by the proteins connexin 30 and connexin 43. Ernst and co-workers measured the levels of connexin 30 and connexin 43 in the prefrontal cortex of suicides, suffering from a range of psychiatric disorders. The graph of the top refer to the results for the messenger RNA of connexin 30 in A and connexin 43 in B. And the graphs at the bottom refer to the protein levels, again of connexin 30 the left and 43 to the right. And the images in the upper right corners of the top graph illustrate semi- quantitative PCR data from control and suicide case.

What is apparent from these graphs is that both, the level of messenger RNA of connexin 30 and connexin 43, and the level of connexin 30 and connexin 33 proteins are reduced in the suicide completers versus control subjects, and this suggests a dysfunction of astrocytic networks in depressed individuals.

Slide 18

Based on the findings of the pilot study on suicide completer shown in the previous slide, the level of connexin messenger RNA and protein were then examined in rats that had been exposed to chronic unpredictable stress, which as we have seen is a model of depression. As in humans, the levels of messenger RNA and protein of both connexins were found to be decreased in the prefrontal cortex of stressed rats.

In the panelling A, we have the results for protein levels. In the panel on the right, we have the result for messenger RNA levels. The protein levels were determined by western blot, and in A, we have representative western blot images for connexin 43 and beta-actin, used as a loading control.

The first three column in A, presents results from controlled rats, either untreated or treated, with two different antidepressants, and columns four to six present results from rats exposed to chronic unpredictable stress and treated in column four or treated with two different antidepressants in columns five and six. Results are presented in the same order for messenger RNA level in C.

What is apparent, is that, in stressed rats, both protein and messenger RNA level for connexin 43 were significantly decreased. The levels were restored by treating with either of the two antidepressants, either Fluoxetine or Duloxetine. It is apparent that both the level of connexin 43 protein and the level of connexin 43 messenger RNA were significantly reduced in chronic unpredictably stressed rast. At the same time, both the level of connexin 43 protein and the level of connexin 43 messenger RNA were restored to normal by antidepressant treatment.

Slide 19

What are the consequences of the decreased level of connexin protein?

Lucifer yellow, which is dye, was injected into the rat brains, as illustrated here. As you can see from the graphs at the top, which are quantifications of the image at the bottom, both the diffusion distance and the number of coupled cells were reduced by chronic unpredictable stress, and this effect was reversed by antidepressant treatment.

In the micrograph at the bottom, we see in CTR, CF, and CD are three control groups: untreated on the left or treated with the two antidepressant on the right. And we can see that the distance of diffusion of the dye, in green, is similar in all three panels.

The bottom left panel, CUS, shows a much reduced, both distance of diffusion and number of couple cells, which is restored to normal in SF and SD, which are the two chronic unpredictable stress group with antidepressant treatment.

Slide 20

Could the decreased number of gap junctions and a reduced coupling of astrocytes have a causative effect in depression? The authors infuse the chemical carbenoloxone, which blocks gap junction in the prefrontal cortex. The asterisk in A indicates the point of the infusion.

Injection of carbenoloxone in the prefrontal cortex induced depressive-like behaviour in rats. This was measured by using the sucrose preference test, which we've already spoken earlier. The first column is animal treated with Vehicle, and shows the effect of carbenoloxone infusion on the sucrose preference test.

Animals that had a control infusion of phosphate-buffered saline, displayed a preference for sucrose-containing water. This was decreased, indicating depressive-like symptoms already at the lowest dose of carbenoloxone. But with increased doses, the depressive symptoms became more apparent.

Slide 21

To summarise the studies described here, we've seen how decreased level of connexin, both connexin 30 and connexin 43, which are the components of gap junction in astrocytes, were identified in both major depressive disorder and in experimental stress.

Furthermore, both Fluoxetine and other antidepressant can reverse these changes in animal models.

We've also seen how blocking gap junction in the prefrontal cortex is sufficient to induce depressive behaviour in animals, suggesting that astrocytic dysfunction may be sufficient to induce the onset of depression. However, the mechanism whereby dysfunctional astrocytic networks may affect mood have not been established yet.

We then hypothesised that decreased expression of connexin 30 and connexin 43 may alter calcium wave propagation and communication between astrocytes, possibly leading to a decrease in the simultaneous release of gliotransmitter and/or affecting the metabolic roles of astrocytic networks, such as potassium buffering, supply of energy substrate, et cetera. Therefore, astrocytic network dysfunction may play a role in depression. But what about gliotransmission?

Whilst the role for gliotransmission in depression can be hypothesised, since disruption of astrocytic networks could affect neurotransmission, currently we have no direct evidence to support this assumption. On the other hand, some recent study on sleep deprivation and depression seem to support the idea, overall, for gliotransmission in depression.

As you may remember, from part one, astrocytes have been shown to play a role in the regulation

of sleep via SNARE-dependent signalling, which is mediated through the adenosine A1 receptor on neurons.

Sleep deprivation is a potent short term antidepressant, which is effective in approximately 60 per cent to 70 per cent of the patient. Sleep deprivation, in the form of one or more nights of total sleep deprivation, can rapidly alleviate symptoms of depression. Interestingly, in learning what happens in human depressed patient, sleep deprivation can reduce depressive-like symptoms in mouse models of depression. But this reduction in depressive-like symptoms is not observed in mice in which the vesicular release of gliotransmitter is prevented by genetic manipulation, or if adenosine signalling is prevented using an adenosine receptor antagonist, or knocking out the A1 Receptor gene.

This suggest that anti-depressive effect of sleep deprivation require gliotransmitter release from astrocytes. This suggests an involvement of gliotransmission in depression.

Slide 22

So astrocyte pathology may play a role in depression, but what causes astrocyte pathology in depression? Stress, acting on the HPA axis, may be a causative factor in depression, and acute and chronic stress may alter astrocyte morphology and physiology. Most of this alteration can be prevented by antidepressant and other protective treatment. Further, correlative evidence supports a role for astrocytes in most, if not all, psychiatric disorder, and you can review some of the evidence in the key readings.

Slide 23

In conclusion, much circumstantial evidence supports the hypothesis, overall, for astrocytic dysfunction in psychiatric disorder. And this evidence comes from a range of studies, as we have seen, from studies in humans to studies in cell culture. Often, the most obvious astrocytes pathology appears to be astrocytic atrophy.

It is currently impossible to determine whether astrocytic pathology is the primary cause of psychiatric disorders, such as depression or schizophrenia, or whether such pathology always emerges as a consequence of neuronal dysfunction - that is, it is secondary to neuronal pathology. Even if genetic studies would suggest that, at least in some cases, astrocytic pathology may be the primary cause, since in some animal models genetic alterations, which are limited to the astrocytes, are sufficient to generate mood disturbances, and you can find evidence of these in the section on schizophrenia.

Targeting astrocytic pathology appears, in some cases, to be sufficient to ameliorate behavioural disturbances, regardless of whether astrocytic pathology is the primary cause of the disorder or secondary to neuronal dysfunction.

Since a considerable body of evidence now suggests that neurons are not the sole determinants of behaviour, it seems fundamental that future research into the neurobiology of psychiatric disorder shifts this focus from a neurocentric perspective to, at least, a neuroglial one, if not affirming a gliocentric one, as suggested by some researchers.

