Environmental influence on biomarkers of depression and anxiety in the hippocampus in an animal model of depression

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Abstract

Animal models have been used extensively to investigate depression and anxiety. In humans, anxiety and depression are typically twice as prevalent in women versus men; however the large majority of studies using animal models are based on male rats. This study addresses this issue by studying two strains of female rodents. As a continuation of a previous study on the influence of environmental manipulation in depressive-like behaviour, this study examines the difference in biomarkers related to depression and anxiety in the brains of an animal model selectively bred for depressive-like symptoms, the Wistar Kyoto rat, and its control counterpart, the Wistar rat. Before sacrifice, the animals were housed in three different environments for four weeks; standard (three animals per cage with no physical enrichment), enriched (six animals with physical enrichment) and isolated (one animal per cage without physical enrichment). Biomarkers for glucocorticoid receptors, microglia and astroglia were investigated in the hippocampus. **Heightened stress levels, resulting from depression and anxiety, are believed to disrupt the functioning of the HPA axis altering the concentration of glucocorticoid receptors.** The brains of the tested rodents were isolated and 16µm sections were used for immunohistochemical analysis. Immunohistochemistry was used to label the biomarkers of interest using antibodies and fluorescence microscopy was used to image the brain tissue. Much work is still necessary to determine the influence of environment on the treatment of depression and anxiety. Further animal studies may lead to increased knowledge for clinical application in humans.

Introduction

Depression and Anxiety

Depression and anxiety are debilitating and prevalent mental disorders, making them the topic of many animal studies. Depression and anxiety have **high rates of co-occurrence**, suggesting related biological mechanisms (Barlow et al 2015).

Biological Background

It has been suggested that both depression and anxiety are result of an overactive neurobiological response to stressful life events or chronic stress (Barlow et al 2015).

The **Hypothalamic-pituitary-adrenal (HPA) axis** regulates secretion of cortisol. **Cortisol** (a glucocorticoid) is a hormone released from the adrenal cortex in response to stress and is responsible for mounting the physiological stress response (i.e. increased heart rate, fight of flight response). The HPA axis has many feedback inputs to regulate the amount of circulating cortisol. One such feedback loop involves the **hippocampus**. Cortisol binds to **glucocorticoid receptors** in the hippocampus, which sends signals back to the HPA axis to stop production of more cortisol (negative feedback regulation) (Bear et al 2007). In other words, a dysregulation in the number of glucocorticoid receptors in the hippocampus can directly affect the amount of circulating cortisol, and has been implicated in biological models of anxiety and depression.

Environmental Influence

Studies suggest an integrative role of biological vulnerability and environmental influences on the development of depression and anxiety (Barlow et al 2015).
Social support and physical activity can have a positive influence on mild to moderate depression. This study aims to see if they will also help in an animal model of 'clinical' depression at the biological level. We are also trying to see what kind of biological influence environmental enrichment or impoverishment can have.

Main Question

Can environmental manipulation influence the concentration of biomarkers of depression and anxiety in the hippocampus in an animal model of depression?

Methodology

Subjects:

- Wistar rats → control
- Wistar Kyoto rats → animal model of depression
- All female rats were used in this study

Environments:

- Standard environment → three animals per cage without physical enrichment
- Enriched environment → six animals per cage with physical enrichment
- Isolated environment → one animal per cage without physical enrichment

Following behavioural studies:

- Subjects were sacrificed
- Brains were isolated, perfused and flash frozen
- Brains were sectioned into 16µm coronal slices
- Slices containing hippocampal regions were then used for immunohistochemical analysis

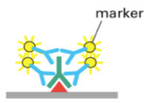
Immunohistochemistry:

An experimental technique used for staining molecules of interest using antibodies. Primary antibodies bind to specific antigens expressed on the molecule of interest, secondary antibodies containing a fluorescence marker then bind to the primary antibodies allowing for visualization.

Application of primary antibodies
- GR → glucocorticoid receptors
- GFAP → astroglia

Incubation – 24 hours

Application of secondary antibodies
- GR → fluoresces green
- GFAP → fluoresces red



Incubation – 2 hours

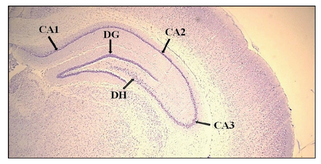
Application of Hoechst
- Labels cell nuclei

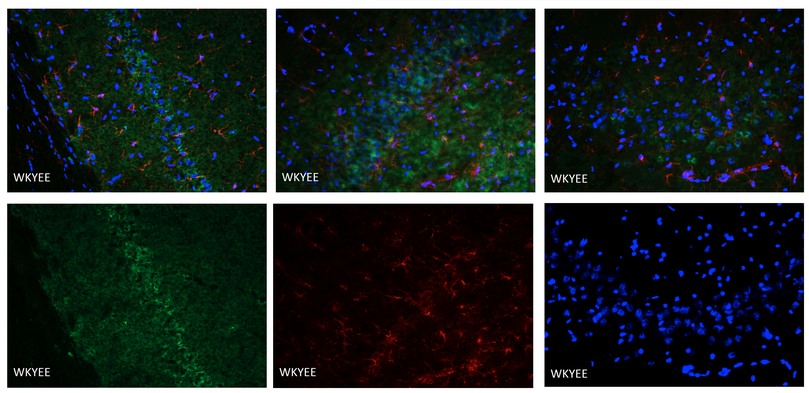
Incubation – 10 minutes

Imaging by Fluorescence microscope

Fluorescence Images

Regions of Interest:
- CA1
- DG
- CA3





CA1 DG CA3

Figure 1. Images taken by a fluorescence microscope following staining by GR (glucocorticoid receptors - green) and GFAP (astroglia - red) antibodies and Hoechst (cell nuclei - blue). All the above images were obtained from Wistar-Kyoto rats (WKY), our animal model of depression, housed in an enriched environment (EE). Photos taken at 20x magnification.

What's Next

Next steps

Immunohistochemistry will be performed on the rest of the rat brain slices (n= 36). Also labelling of microglia using another set of primary and secondary antibodies will be performed. When all labelling is complete the brain tissue will be viewed under the fluorescence microscope. The pictures obtained will be further analyzed using software capable of determining relative quantities of biomarkers of interest.

Expectations

Comparisons will be drawn between the Wistar and Wistar-Kyoto rats within each of the varying environments (standard, enriched and isolated). It is expected that the animal model of depression, Wistar-Kyoto rats, will display a **lower concentration of glucocorticoid receptors** in comparison to the control group, Wistar rats. Further it is anticipated that Wistar-Kyoto rats will show a **higher level of glucocorticoid receptors** (displaying a lower activation of the HPA axis in response to stress) in an **enriched environment** rather than an isolated environment.

Environmental Influences

If the outcome of this project is as hypothesized, it may suggest that an **enriched environment can have a positive influence** on depression and anxiety. The findings would offer support to the idea that manipulation of the environment may alter an individual's response to stress at a biological level. Further experimental support to the interaction of biology and environment in mental illness will contribute to potential **future treatments and interventions** in people who suffer from depression or anxiety.

References

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