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Schizophrenia, fats and lab rats

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Last year I joined a dedicated research group on a quest to discover what causes schizophrenia. My honours project was designed to investigate whether fats, as part of the structure of cell membranes, could alter neurotransmitter function in the brain and potentially play a role in the disease. I found that fatty acids can influence neurotransmitter receptor binding in the rat brain, and may play some role that can be related to schizophrenia. In this article I provide an introduction to the science of schizophrenia and discuss new techniques that are available to study the disorder and insights into my own research findings.
Introduction

What is schizophrenia? Schizophrenia is a mental illness that has puzzled scientists for many decades, and still remains largely unresolved as to its cause. It affects approximately one in every hundred people, and is a significant burden on health expenditure in Australia. The illness interferes with mental functioning and, in the long-term, may cause changes to a person’s personality. The consequences of having schizophrenia can be massive, both on the sufferer and their family (for example, see the recent media attention on the Baxter detainee, Cornelia Rau). Symptoms include hallucinations, delusions and thought disorganisation, as well as impaired motivation and decreased emotional expression.

It is generally accepted that schizophrenia involves imbalances in signalling chemicals of the brain called neurotransmitters. Neurotransmitters are proteins that carry information from cell to cell, or as they are called in the brain, neuron to neuron. These chemicals form part of pathways that can inhibit or excite neural cells. An imbalance of these pathways may contribute to symptoms of schizophrenia by changing the amount of activity in certain brain regions, and even by causing cell death, as too much excitation can result in neurotoxicity. Many, if not all brain regions seem to be involved in schizophrenia, which is not surprising given the interconnectivity between pathways and systems.

In the past few decades, the most widely studied neurochemical explanation of schizophrenia has been the dopamine hypothesis, which suggests that hyperactivity of the dopamine system is the primary pathology of the disorder. This was based on the fact that antipsychotic drugs, commonly used to treat schizophrenia, have anti-dopaminergic properties. However this hypothesis has failed to produce conclusive findings. For one reason, a significant proportion of schizophrenia patients do not respond to anti-dopamine therapy. Other popular theories include the neurodevelopmental hypothesis, which proposes that schizophrenia may be the result of a chemically based injury to the brain either before or during the critical period of brain development - late adolescence in humans. This period coincides with the age that schizophrenia symptoms begin to appear. During this time the brain undergoes ‘synaptic pruning’, which basically means getting rid of unnecessary cell signalling pathways and enhancing useful ones, making the brain more efficient.

There is also emerging evidence in support of the glutamate N-Methyl-D-aspartate
(NMDA) receptor hypofunction hypothesis. NMDA receptor antagonists such as phencyclidine (PCP, angel dust) and ketamine (special K) can induce the entire range of core schizophrenia phenomenology including disorder of attention, perception and abstract thinking in normal subjects, and can exacerbate symptoms in patients with schizophrenia, while the psychotic effects produced by amphetamine (dopamine transporter inhibitor) and lysergic acid (LSD) (serotonin agonist) do not fully represent the scope of the main symptoms of schizophrenia. Indeed, PCP-induced psychosis is diagnostically difficult to differentiate from schizophrenia. This observation has led many researchers to believe that glutamate NMDA receptors are the key to unravelling the pathology of schizophrenia.

Techniques available for examining the cellular basis of schizophrenia

There are several methods of examining schizophrenia at the cellular level. The post mortem brain of schizophrenia has been examined extensively, with a number of abnormalities detected in many neurotransmitter systems. To measure the density of neurotransmitter receptors, the procedure involves using thinly sliced brain tissue sections that are treated with a radioactively tagged drug that binds to a specific receptor, which is present in the brain sections. To quantify the amount of radioactivity, and therefore the receptor density, sections can be exposed to radioactive-sensitive film for several months, then the film is developed and analysed. Alternatively, sections can be quantified using Beta-Imager Detection. The University of Wollongong has recently installed a BioSpace Beta Imager Machine in the Molecular Neurobiology Laboratory, directed by Associate Professor Xu-Feng Huang. The Beta Imager is unique in that it can immediately measure the distribution of beta particles emerging from brain sections and display them on screen. The image can be directly acquired and quantified within around four hours, and it is therefore much more efficient than film autoradiography. Furthermore, because this is a direct capture of the beta particle signals, it is significantly more accurate. The Beta Imager was obtained through funds raised by the local Illawarra community, championed by Lord Mayor Alex Darling of Wollongong City Council, in partnership with the Neuroscience Institute of Schizophrenia and Allied Disorders (NISAD). This machine is the first of its kind available in the southern hemisphere.

Other new techniques available include functional magnetic resonance imaging (fMRI), where a patient's brain activity is assessed while performing tasks that are known to activate certain brain regions. Recently, using this technique, a team of researchers led by NISAD researchers in NSW, found a direct link between
brain structural abnormalities, and core features of the disorder (www.nisad.org.au). DNA microarray technology is another application of advanced technology allowing thousands of genes to be analysed for abnormalities simultaneously, and has been used to identify abnormal genes in schizophrenia.⁹

Treatment

Symptoms of schizophrenia patients vary significantly. Typical treatment of this disorder involves a lifetime of large doses of antipsychotic drugs, which are only partially effective, expensive, and have many undesirable side effects. Haloperidol, a first generation ‘typical’ antipsychotic drug (produced in 1960) is effective in treating positive symptoms, but it has severe motor side effects. Second generation drugs such as Olanzapine and Clozapine (produced in 1990) are known as ‘atypical’ antipsychotics and are effective in treating both positive and negative symptoms, but can cause severe obesity. A third generation antipsychotic drug Aripiprazole (produced in 2003) is also effective in treating both positive and negative symptoms, and it does not cause obesity.

My study

My honours project was designed to see whether fatty acids could alter neurotransmitter function in the brain, and potentially play a role in the disease. The study was based on three premises: First, post mortem schizophrenia brains show lower levels of certain omega-3 and omega-6 fatty acids in their neural and red blood cell membranes, as well as altered levels of markers for fatty acid metabolism. Second, many neurotransmitter systems are altered in schizophrenia. Third, omega-3 fatty acid supplementation in addition to antipsychotic medication has been shown by some groups to be successful in improving mental health in schizophrenia patients. This result seems to depend on a dose that optimises the omega-3 to omega-6 ratio.¹⁰ I wanted to determine if there was a connection between these findings, that is, is there a relationship between membrane fatty acid status in the brain, neurotransmitter levels and brain function?

But what have fatty acids got to do with neurotransmitters? In short, all cells including neurons are surrounded by a membrane that functions as a filter, allowing only certain materials to pass in and out of the cell. The membrane itself is embedded with various proteins including neurotransmitter receptors. Membranes are comprised of phospholipids, which are made up of different types of fatty acids. It has been shown that rats fed a large percentage of omega-3 fatty acids in their diet have a higher ratio of omega-3 fatty acids in their neural membrane phospholipid composition. The same can be said for other fatty acid types like omega-6 and saturated fats. Further studies have demonstrated
that by manipulating fatty acid content in the diet and therefore in membrane phospholipids, the density of neurotransmitter receptors in the brain can be altered.

In my study, rats were fed diets high in saturated fat, omega-3 polyunsaturated fatty acid (PUFA), omega-6 PUFA or a control diet for 8 weeks. I examined receptor subtypes from the serotonin and acetylcholine muscarinic neurotransmitter systems, both of which have been implicated in schizophrenia in addition to the popular dopamine and glutamate systems. To do this I needed to do a series of drug-binding experiments, involving applying a radioligand, which is a radioactive drug that binds to a certain receptor, to brain sections from the four diet groups. I measured the amount of radioactivity, and therefore the receptor density, using both the traditional film method (Fig. 1) and Beta-Imager technology (Fig. 2).

I analysed areas throughout the whole brain, and made several interesting findings. Compared to rats on a normal laboratory diet, rats on saturated fat, omega-3 and omega-6 diets all showed changes in receptor density, but these were quite varied. The most pronounced effects were seen in the omega-6 fed group, which showed increased muscarinic receptor binding in several brain regions. The results illustrate that diet can alter the density of neurotransmitters in the rat brain. So does this mean that diet can alter the density of neurotransmitters and brain function in schizophrenia are simply due to fatty acid imbalances? Unfortunately it is not so simple. For one reason, abnormalities in fatty acid levels in schizophrenia have been attributable to metabolic abnormalities, and not to a dietary deficiency. It should also be pointed out that abnormal fatty acid levels could be a consequence of changes in neurotransmitter receptor interaction and not vice versa. To bring an old cliché to mind, it appears to be a case of which came first: the chicken or the egg? So while a definitive role for fatty acids in schizophrenia pathology remains uncertain, studies on the influence of fatty acids on neurotransmitter receptors do still have potential to improve the treatment of schizophrenia using dietary fatty acid supplementation.
Fig. 1. Film autoradiography showing the binding pattern of muscarinic M1/M4 and M2/M4 receptor subtypes in the brain of control rats. Note the similar distribution of muscarinic receptor subtypes at the level of A and B, while the distribution of receptor subtypes differs at the level of C. The maps of A to C are adapted from a standard rat brain atlas indicating the levels that sections were cut. A'-C': [3H]pirenzepine binding to muscarinic M1/M4 receptor subtypes; A''-C'': [3H]AF-DX384 binding to muscarinic M2/M4 receptor subtypes. Abbreviations: Acb: nucleus accumbens; ACC: anterior cingulate cortex; Amg: amygdala; AOP: anterior olfactory nucleus; CA1-3: CA1-3 fields of the hippocampus; CPU: caudate putamen; DG: dentate gyrus; PFCx: prefrontal cortex; Tu: olfactory tubercle.
Fig. 2. Beta-lmager detection showing the binding pattern of serotonin $5\text{-HT}_{2A}$ and $5\text{-HT}_{2C}$ receptor subtypes in the brain of control rats. Note the similar distribution of serotonin receptor subtypes at the level of A and B, while the distribution of receptor subtypes differs at the level of C. The maps of A to C are adapted from a rat brain atlas and indicate the levels that sections were cut. A'-C': $5\text{-HT}_{2A}$ receptors; A''-C'': $5\text{-HT}_{2C}$ receptors. Abbreviations: Acb: nucleus accumbens; ACC: anterior cingulate cortex; Amg: amygdala; AOP: anterior olfactory nucleus; CA1-3: CA1-3 fields of the hippocampus; CPU: caudate putamen; DG: dentate gyrus; DM: dorsomedial hypothalamus; LH: lateral hypothalamus; PfCx: prefrontal cortex; Tu: olfactory tubercle; VMH: ventromedial hypothalamus.
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REFERENCES


