Title – Patterns of synaptic pruning predicating pathogenesis of Psychoses, Autism and ADHD? Evidence from neuro-immunology and longitudinal imaging: potential of biomarkers, immunotherapeutic solutions.

Abstract –

This review of available longitudinal structural imaging and immunological findings in first onset schizophrenia, bipolar disorder, attention deficit hyperactivity disorder (ADHD) and autism suggest different patterns of synaptic pruning leading to various phenotypes.

Proposals for future research strategy are suggested to assist replication of findings, along with potential biomarkers to assist diagnosis and duration of maintenance treatment, along with ideas of potential immunotherapy augmentation are suggested.

Learning objectives include understanding the immunological basis of synaptic pruning, comprehending the available research in longitudinal imaging and being aware of future immunological therapeutic strategies in psychosis, ADHD and autism.

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Introduction – why is this topic important?

It is estimated that over 50% of major psychiatric conditions show prodromal symptoms in childhood and adolescence when most synaptic pruning takes place (1). Less than half the presentations lead to diagnosis at the time, resulting in poor prospects regards education, employment and relationships. Currently, there are no biomarkers to delineate the actual diagnosis and to guide treatment selection, and duration of prophylaxis.

Physiology of synaptic pruning

Synaptic pruning plays a crucial role in brain maturation by reducing excessive amounts of synapses developed in utero (2). The process of pruning is activity dependant, using the principle of 'use it or lose it'. Pruning usually takes place during specific age parameters, followed by myelination in order to create more efficient circuits. The initial spell of generalised synaptic pruning occurs between birth and 2 years (Phase 1), followed by a further generalised pruning

during adolescence (Phase 2) from 11 to 15 years with some variability between the sexes (3). The final spell of synaptic pruning is largely focussed on the pre-frontal area of the brain and takes place in early adulthood between 18 and 25 years (Phase 3). These spells appear to be associated with grey matter volume loss in serial imaging which can be considered a proxy measure of the extent of pruning (4).

Pruning is carried out by microglia performing phagocytosis of unwanted synapses (5). However, synapses which are to be eliminated need to be 'tagged' with a protein marker in order to preserve other active circuits. Studies in mice show that rapidly replicating neurones produce a signalling protein called Fractaline, which binds to a receptor Cx3cr1 that is found exclusively in microglia, causing activation (6). Microglia can also be activated in the pre frontal cortex by chronic stress as shown in animal studies (7). Cells supporting neurones - astrocytes - also assist synaptic pruning by identifying synapses primed for elimination (8). Astrocytes secrete a cytokine termed TGF-beta which activates the complement cascade; C1q, C4, C2 and finally C3 (9), again leading to microglial activation.

Microglial activity is measured via Positron Emission Tomography (PET) using radioactive ligands binding to

benzodiazepine receptors (10). More recently, quantitative analysis of microglial activation has been achieved by using the ligand PK11195, another proxy measure of pruning (11).

It is unclear is why there are recurrent spells of pruning; if onset is a threshold effect mediated by synaptic load, or a response to social, hormonal or genetic signals. There appears to be variable amounts of pruning in different parts of individual brains; again the reason is unclear, possibly associated with differential brain usage by individuals.

Early onset psychiatric disorders - 3 syndromes.

Persisting, recurrent or brief psychotic illnesses in the absence of gross brain abnormality are called 'functional' psychoses (12). Across the world it is estimated that 1% of the adult population experience these syndromes. These include Schizophrenia, Bipolar disorder and a 'hybrid' psychosis called schizoaffective disorders; a mixture of symptoms seen in schizophrenia and bipolar disorder. These disorders show 'prodromal' features in adolescence such as attentional and memory difficulties as well as

sequencing problems along with affective changes or apathy. The onset of acute symptoms such as mania, delusions and hallucinations occur in early adulthood, often in the context of major life changes or substance misuse. It is also not uncommon for patients with psychoses to have experienced severe stress (for example sexual abuse) in their childhood, which is known to heighten immune responsivity (13).

A modal of how immune processes can cause psychosis has emerged via encephalitic conditions presenting with psychosis with antibodies to NMDA receptors or voltage gated potassium channels (14). This process appears to explain around 6% of acute psychoses. These syndromes are resistant to anti-psychotics but sensitive to steroids and other anti-inflammatory agents. Recurrences are common, but treatable with immunotherapy including plasmapheresis to clear the antibodies from circulation. Furthermore, there has also been resurgence of interest in the association between increased risks of schizophrenia in the context of prenatal infections (15), which can cause kindling of the foetal complement cascade via the transfer of maternal IgG (16).

Autistic Spectrum Disorder (ASD) also presents in childhood in around 1/150 children, with a combination of inattention, sequencing difficulty, and behaviour patterns which appear to be present to cope with difficulty in establishing and maintaining social communication and emotional closeness to others (17). Often there are behavioural rituals, which if prevented, can result in a display of severe anxiety. ASD is also associated with various compulsive disorders, including self-harming, obsessional checking, vocalisations etc.

Attention Deficit Hyperactivity Disorder (ADHD) (18), which affects 3-5% of children, is a condition with inattention and sequencing problems. Boys tend to show more hyperactive features, with girls showing more inattention. There is a tendency to display risky behaviours as part of impulsivity.

Evidence of Pruning in Psychoses

Thompson and colleagues (19) serially scanned adolescents with schizophrenia, alongside their healthy siblings as well as matched controls. They found that there was a 4 fold excess of permanent grey matter loss in children who developed schizophrenia

compared to controls. There was also evidence of excess grey matter loss in siblings, which subsequently ameliorated (20). Furthermore, a recent Positron Emission study found excess microglial activity in patients with schizophrenia and, to a lesser extent, in people with high risk of the disease compared to controls (21).

Immunological research in schizophrenia has shown that the complement cascade is altered with C1, C3 and C4 showing increased mean activity in patients with schizophrenia, with C2 showing reduced activity (22). A further study showed increase in interleukin1 beta (an inflammatory marker) in patients with schizophrenia (23). The latest finding is an allele of the C4 gene (C4A) more likely in patients with schizophrenia (24).

On Bipolar illness, recent serial MRI scanning of adolescents (25) shows that those proceeding to a bipolar illness or psychosis with mood lability (schizoaffective disorder) appeared to show excessive grey matter loss involving the bilateral anterior and subgenual cingulate cortex rather than generalised grey matter loss. Major Depressive Disorder (MDD) has been associated with increased microglial activation in the cingulate gyrus (26) with a trend towards this finding in bipolar depression.

Evidence in Autism

Evidence points to an acceleration of brain growth during the early years in autism. Courchesne and colleagues (27) have demonstrated that children with autism show lower than expected brain weight at birth, thereafter changing to excess brain weight during the next 3 years, involving both grey and white matter in all brain regions except occipital grey matter, with the most growth demonstrated in the frontal, temporal and cingulate areas. Diffusion Tensor imaging of young children with autism (28) suggests increased axons and myelination between neighbouring areas of the brain compared to more distal connections. Furthermore, there has been evidence of increased density of activated microglia in the dorsolateral prefrontal cortex in autism (29), but none elsewhere.

Attention Deficit Hyperactivity Disorder.

Rapport and colleagues (30) compared serial imaging findings of children with attention deficit disorder (ADHD) with those with childhood onset schizophrenia. They found that the degree of grey and white matter loss in ADHD children was less than in that in schizophrenia. Grey and white matter losses were in

the dorsolateral pre frontal cortex, caudate, pallidum, corpus callosum and cerebellum (31).

Current explanations

Currently the causative mechanisms to explain ADHD, Bipolar disorder and Autism with back up evidence is not available, apart from the evidence of family history increasing risk via multiple genetic routes. There is no evidence that immune reactions to vaccinations play a role in any of these conditions.

Schizophrenia is the only condition where there has been an evidenced based theory; of dopamine and glutamate dysregulation, i.e. excess dopamine supply to the striatal areas due to reduced inhibition of this process caused by pre frontal inactivity and subsequent reduction in glutamate supply to the striatum (32). The reason for frontal inactivity has not been elucidated so far. From a genetic perspective, Harrison and Weinberger (33) have provided an overview of how the various putative susceptibility genes (such as DISC1, Neuregulin, Dysbindin) could interact to produce neuropathology in terms of synaptic pruning.

Potential common pathogenic pathway

On the basis of above evidence, a parsimonious explanation of pathogenesis of all these disorders is increasingly discussed by researchers in the field, albeit without peer reviewed publications apart from a single paper on schizophrenia (34). The idea is of differing patterns of synaptic pruning; either being excessive at all phases in Schizophrenia, excessive in some brain regions in Bipolar disorder (consistent with the spectrum of clinical presentation between schizophrenia and bipolar disorder); inadequate at all phases in Autism, low grade but persistent across all of childhood in ADHD (again accounting to a spectrum and/or co-morbidity of these 2 conditions). It is also being considered that unipolar major depression is due to stress induced 'reactive' synaptic pruning in frontal and cingulate areas.

Data interpretation problems and future strategy

The main problem with the available evidence is low subject numbers in each study, typically under 20 in each arm, and the lack of replication of positive findings. It is likely that publication bias has limited negative findings. It also has to be noted that the

positive findings are simply group mean differences. Furthermore, serial scanning of children and adolescents, especially using radioactive ligands, is a limiting factor. However, imaging of children in the early stages of illness carries less likelihood of confounding due to prolonged psychotropic use, both illicit and prescribed. Replication of the above positive findings would be helpful using the same inclusion criteria, and controlling for psychotropic use.

Brain volume assessment and measuring regional ligand based perfusion tends to vary between different imaging centres; there needs to be multicentre studies with a single imaging protocol and central evaluation of findings. Similar issues exist in biochemical analysis of complement activity.

Therefore future strategy involves a multi-centre longitudinal MRI study of ‘at risk’ adolescents of schizophrenia, bipolar disorder, ADHD and Autism compared to siblings and age and sex matched controlled with annual scanning over 2 years. Furthermore, a study of activated microglia following diagnosis, looking at effects of time, psychosocial

intervention and medication needs to be carried out with both imaging techniques and subsequent reporting controlled centrally

Potential for Biomarkers and immunotherapy

Immunological findings could be utilised as bio-markers of active disease and prognostic indicators of chronicity. In particular, direction of travel of inflammatory markers (especially complement parameters) could help judge duration of maintenance treatment to avoid relapse in psychosis and ADHD. This is the main area of investigation currently, involving microglial and complement activity.

Furthermore, a combination of serial grey matter volume studies and extent of microglial or complement activation might predict outcome in first episode psychosis, and assist decisions on anti-psychotic prophylaxis. Similarly, continuing asymmetry of grey matter volume in bipolar disease and lack of grey matter growth in ADHD could inform maintenance therapy regimes.

Regards immunotherapy, it is possible to develop drugs which increase neuronal pruning by activating the complement system, and drugs which limit pruning by reducing glial activity. Rapamycin, used in immunosuppression after transplantation does enhance neuronal pruning, as evidenced in rats (35). This drug is toxic to humans, but variants of the molecule might be useful in autism, even after emergence of symptoms as found in animal studies.

Minocycline, an antibiotic and anti-inflammatory agent, has been shown to reduce microglial activation, and has shown early promise as an augmenter of antipsychotics in both negative and cognitive symptoms in schizophrenia (36). In vitro studies of some antipsychotic agents also show attenuation of microglial activation via cytokine production (37). Lithium also appears to have an effect in reducing microglial activation via the P13K / Akt intracellular signalling pathway (38), suggesting a strong rationale for continued use in prophylaxis.

The potential of increasing activated microglia via bone marrow transplantation has been confirmed using

mouse models of Rett's syndrome (39), which is similar to autism. Similar to treating fluid tumours (40), the body's pre-microglia cells could be removed from marrow, grown and activated in vitro before being returned to help the pruning process. Peripheral infusion is possible, as microglia migrate to the brain (41). Finally, the finding of an excess of a C4 allele in schizophrenia could suggest gene silencing of this protein (42).

Conclusion

Combining immunological findings with longitudinal structural imaging in childhood psychiatric diseases lends itself to a unified hypothesis, with real world clinical benefits in screening and treatment.

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MCQ's (select the False item)

1. Processes of neuronal synaptic pruning includes
   a. GLUT transporters (F)
   b. Microglia
   c. Astrocytes
   d. Complement cascade
   e. Fractaline
2. Encephalitic psychoses
   a. Make up 6% of psychoses
   b. Are linked to potassium channel antibodies
   c. Linked to NMDA receptor antibodies
   d. Linked to calcium channel antibodies (F)
   e. Resistant to antipsychotics
3. Autistic Spectrum Disorder
   a. Lower than expected brain weight at birth
   b. Is associated with excess brain weight in first 3 years of life
   c. Increased connectivity between distant areas of brain (F)
   d. Increased connectivity between local brain areas
   e. Increased density of activated microglia in dorsolateral prefrontal cortex
4. In childhood onset schizophrenia
   a. A fourfold increase in grey matter loss is found compared to controls
   b. Reduced microglial activation (F)
   c. Complement C1, C3, C4 is increased
   d. Complement C2 is reduced
   e. C4A allele is increased in prevalence
5. Proposed Immunotherapeutic approaches include
   a. Minocycline
   b. Ropinarole (F)
   c. Plasmapheresis

d. Steroids
e. Microglial activation in vitro