ARTICLE

Do patterns of synaptic pruning underlie psychoses, autism and ADHD?*

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SUMMARY

This review of available longitudinal structural imaging and immunological findings in first-onset schizophrenia, bipolar disorder, attention-deficit hyperactivity disorder (ADHD) and autism suggests that different patterns of synaptic pruning lead to various phenotypes. Proposals for future research strategies to try to replicate these findings are suggested, potential biomarkers to assist in diagnosis and determining the optimum duration of maintenance treatment are considered and ideas of potential immunotherapy augmentation are outlined.

LEARNING OBJECTIVES

- Understand the immunological basis of synaptic pruning
- Comprehend the available research on longitudinal brain imaging
- Be aware of future immunological therapeutic strategies in psychosis, ADHD and autism

DECLARATION OF INTEREST

None.

It is estimated that over 50% of major psychiatric conditions show prodromal symptoms in childhood and adolescence, when most synaptic pruning takes place (Kim-Cohen 2003). Less than half of presentations for these symptoms lead to diagnosis at the time, resulting in poor prospects as regards education, employment and relationships. Currently, there are no biomarkers to inform the specific diagnosis or to guide treatment selection and duration.

Physiology of synaptic pruning

Synaptic pruning plays a crucial role in brain maturation by reducing excessive amounts of synapses developed *in utero* (Chechik 1998). The process of pruning is activity dependent, using the principle of 'use it or lose it'. Pruning followed by myelination to create more efficient circuits usually takes place during three age-related phases between birth and early adulthood (Box 1). Serial magnetic resonance imaging (MRI) has revealed that these phases appear to be associated with grey matter volume loss, which can be considered a proxy measure of the extent of pruning (Sowell 2001).

Pruning is carried out by microglia performing phagocytosis of unwanted synapses (Trapp 2007). However, to preserve other active circuits, synapses which are to be eliminated need to be 'tagged' with a protein marker. Studies in mice show that rapidly replicating neurons produce a signalling protein called fractalkine, which binds to the receptor CX3CR1, which is found exclusively in microglia, causing activation (Jones 2010).

Animal studies have shown that microglia can also be activated in the prefrontal cortex by chronic stress (Wohleb 2012). The relationship between stress and microglial activation is based on the release of cytokines such as interleukin-6 (IL-6), induced by stressors. IL-6 penetrates the blood–brain barrier and activates microglia in the central nervous system (Liu 2012). Furthermore, IL-6 plays a key role in pathogenesis of mood disorders.

Microglial activity can be measured via positron emission tomography (PET) using radioactive ligands binding to benzodiazepine receptors (Banati 2002). More recently, quantitative analysis of microglial activation has been achieved using the ligand PK11195, another proxy measure of pruning (van Berckel 2008).

BOX 1 The three phases of synaptic pruning

Phase 1 Initial generalised synaptic pruning between birth and 2 years

Phase 2 Further generalised pruning during adolescence (11–15 years of age), with some variability between the genders

Phase 3 The final spell of synaptic pruning is largely focused on the prefrontal area of the brain in early adulthood (18–25 years of age)

(Jetha & Segalowitz, 2012)

Cells supporting neurons – astrocytes – also assist synaptic pruning by identifying synapses primed for elimination (Chung 2013). Astrocytes secrete a cytokine named transforming growth factor beta (TGF-β), which activates the complement cascade C1q, C4, C2 and finally C3 (Fishelson 2001; Banati 2002), again leading to microglial activation.

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

Early-onset psychiatric disorders: three syndromes

Persisting, recurrent or brief psychotic illnesses in the absence of gross brain abnormality are called 'functional' psychoses (Murray 1997). 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, which is a mixture of symptoms seen in schizophrenia and bipolar disorder. These disorders usually show 'prodromal' features in adolescence such as attentional and memory difficulties, as well as sequencing problems and affective changes or apathy. The onset of acute symptoms such as mania, delusions and hallucinations generally occur in early adulthood, often in the context of major life changes or substance misuse. It is also not uncommon for individuals with psychoses to have experienced severe stress (for example sexual abuse) in childhood, which is known to heighten immune responsivity (Ayaydin 2016).

Psychoses

A model of how immune processes can cause psychosis has emerged via encephalitic conditions presenting with psychosis in which antibodies to *N*-methyl-D-aspartate (NMDA) receptors or voltage-gated potassium channels have been present (Shah 2013). Immune processes appear to explain around 6% of acute psychoses. Antibodies antagonising NMDA receptors can affect the hippocampus, causing hallucinations, paranoia and cognitive impairment (Mak 2009a).

These syndromes are resistant to antipsychotics 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 been resurgence of interest in the association between increased risk of schizophrenia in later life and prenatal infections (Brown 2010), which can cause kindling of the fetal complement cascade via the transfer of maternal IgG antibodies (Palmeria 2012).

Autism spectrum disorder

Autism spectrum disorder (ASD) presents in childhood in around 1/150 children across the world, with a combination of inattention, sequencing difficulty and behavioural patterns that appear to be present to cope with difficulty in establishing and maintaining social communication and emotional closeness to others (Chawarska 2007). 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 and vocalisations.

Attention-deficit hyperactivity disorder

Attention-deficit hyperactivity disorder (ADHD) (Kooij 2010), which affects 3–5% of children across the world, 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 (2001) serially scanned the brains of young adolescents with schizophrenia, alongside their healthy siblings and matched controls, over a 5-year period. They found a fourfold excess of permanent grey matter loss in the children with schizophrenia compared with controls. There was also evidence of excess grey matter loss in siblings, which subsequently ameliorated (Mattai 2011). Furthermore, a recent PET study found excess microglial activity in people with schizophrenia and, to a lesser extent, in people at high risk of the disease compared with controls (Bloomfield 2015).

Immunological research in schizophrenia has shown that the complement cascade is altered, with C1, C3 and C4 showing increased mean activity in people with schizophrenia, whereas C2 shows

BOX 2 Complement cascade

The complement cascade is a biochemical process involving the C1 to C9 complement proteins in which one protein interacts with another in a specific sequence. C5b with C6, C7, C8, and C9 form the membrane attack complex that initiates cell lysis. Other molecules, such as C3a and C5a, act as cytokines, leading to inflammation.

reduced activity (Hakobyan 2005). A further study showed increase in interleukin 1 beta (IL-1β, an inflammatory marker) in patients with schizophrenia (Soderland 2009). The latest finding is an allele of the *C4* gene (C4A) that is more likely to be present in patients with schizophrenia (Sekar 2016).

In bipolar disorder, serial MRI scanning of adolescents (Gogtay 2007) found that those proceeding to a bipolar illness or psychosis with mood lability (schizoaffective disorder) appeared to show excessive grey matter loss in the bilateral anterior and subgenual cingulate cortex rather than generalised grey matter loss. Major depressive disorder has been associated with increased microglial activation in the cingulate gyrus, a finding that is also being reported in bipolar depression (Steiner 2011).

Evidence in autism

Evidence points to an acceleration of brain growth during the early years in autism. Courchesne and colleagues (2003) have demonstrated that children with autism have lower than expected brain weight at birth, thereafter changing to excess brain weight during the next 3 years; both grey and white matter in all brain regions except occipital grey matter are involved, with the most growth demonstrated in the frontal, temporal and cingulate areas (Schumann 2010). Diffusion tensor imaging of young children with autism (Walker 2012) suggests increased axons and myelination between neighbouring areas of the brain compared with more distal connections. Furthermore, there has been evidence of increased density of activated microglia in the dorsolateral prefrontal cortex, but not in other brain regions (Walker 2012).

Attention-deficit hyperactivity disorder

Rapoport and colleagues (2001) compared serial MRI findings from children with ADHD with those from children with childhood-onset schizophrenia. They found that the degree of grey and white matter loss in ADHD was less than that in schizophrenia. Grey and white matter losses were in the dorsolateral prefrontal cortex, caudate, pallidum, corpus callosum and cerebellum (Durston 2003).

Current explanations

At present, the only well-evidenced causative mechanism to explain ADHD, bipolar disorder and autism is that family history increases risk of these disorders 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 of the three for which where there has been an evidenced-based theory, that of dopamine and glutamate dysregulation, i.e. there is excess dopamine supply to the striatal areas due to reduced inhibition of this process caused by prefrontal inactivity and subsequent reduction in glutamate supply to the striatum (Stone 2007). The reason for frontal inactivity has not yet been elucidated. From a genetic perspective, Harrison & Weinberger (2005) have provided an overview of how the various putative schizophrenia susceptibility genes (such as *DISC1*, *NRG1* and *DTNBP1*) could interact to produce neuropathology by way of synaptic pruning.

Potential common pathogenic pathway

A parsimonious explanation of the pathogenesis of all these disorders based on the above evidence is increasingly discussed by researchers in the field, albeit without peer-reviewed publications apart from a single paper on schizophrenia (Keshavan 1994). The idea is that there are differing patterns of synaptic pruning: it is excessive in all brain regions in schizophrenia and also excessive in some brain regions in bipolar disorder (consistent with the spectrum of clinical presentation shared by schizophrenia and bipolar disorder); and it is inadequate at all phases in autism, and low grade but persistent across all of childhood in ADHD (again accounting for a shared spectrum of clinical presentation and/or comorbidity of these two conditions). It is also being considered that unipolar major depression is due to stress-induced 'reactive' synaptic pruning in frontal and cingulate areas potentially at any age.

Data interpretation problems and future strategy

The main problem with the available evidence is the small samples in each study, typically fewer than 20 in each arm, and the lack of replication of positive findings. It is likely that publication bias has limited the publication of 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 positive findings mentioned in this article would be helpful using the same inclusion criteria and controlling for psychotropic use.

Brain volume assessment and measurement of regional ligand-based perfusion tend to vary between different imaging centres; there is a need for multicentre studies with a single imaging

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protocol and central evaluation of findings. Similar problems exist in biochemical analysis of complement activity.

Thus, future strategy should involve a multicentre longitudinal MRI study of adolescents at risk for schizophrenia, bipolar disorder, ADHD or autism compared with siblings and age- and gender-matched controls, 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 subsequent reporting controlled centrally.

Potential for biomarkers and novel interventions

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

A combination of serial grey matter volume studies and studies of the extent of microglial or complement activation might predict outcome in first-episode psychosis and assist decisions on antipsychotic treatment duration. Similarly, continuing asymmetry of grey matter volume in bipolar disorder and lack of grey matter growth in ADHD could inform maintenance therapy regimes.

As regards immunotherapy, it is possible to develop drugs that increase neuronal pruning by activating the complement system and drugs that limit pruning by reducing glial activity (Box 3). However, immunotherapy can cause side-effects, including leukopenia and infection (Mak 2009b). Therefore, the prescription of immunotherapy should be based on a risk–benefit analysis and patient- and disease-related factors.

Rapamycin, used in immunosuppression after transplantation, enhances neuronal pruning, as evidenced in rats (Sato 2012). 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 to treat negative and cognitive symptoms in schizophrenia (Levkovitz 2010). *In vitro* studies of some antipsychotic agents also show attenuation of microglial activation via cytokine production (Bian 2008). Lithium also appears to have an effect in reducing microglial activation via the P13K/Akt intracellular signalling pathway (Dong 2014), suggesting a strong rationale for its continued use in treatment.

The potential of increasing activated microglia via bone marrow transplantation has been confirmed using mouse models of Rett syndrome (Derecki 2012), which is similar to autism. Analogous to the treatment of blood cancers (Davila 2014), microglia at an early stage of development could be removed from marrow regardless of a person's age, grown and activated *in vitro* before being returned to help the pruning process. Peripheral infusion of activated microglia is possible, as microglia migrate to the brain (Grossmann 2002).

Finally, the finding of an excess of a C4 allele in schizophrenia could suggest gene silencing of this protein (Liu 2011).

BOX 3 Potential immunotherapy for synaptic under- or overpruning

- Rapamycin (immunosuppressant) enhances neuronal pruning
- Minocycline (antibiotic and anti-inflammatory) reduces microglial activation
- Atypical antipsychotics such as perospirone, quetiapine and ziprasidone attenuate microglial activation via cytokine production
- Lithium reduces microglial activation via the P13K/Akt intracellular signalling pathway
- Bone marrow transplantation to increase activated microglia
- Plasmapheresis to clear the antibodies from bone marrow or circulation
- Peripheral infusion of activated microglia
- Gene silencing, for example of the C4 allele in schizophrenia

MCQ answers
1 c 2 b 3 e 4 e 5 b

Conclusions

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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MCQs

Select the single best option for each question stem

1 Processes of neuronal synaptic pruning involve:
a GLUT transporters
b dopamine transporters
c astrocytes
d leukocytes
e oligodendrocytes.

2 Encephalitic psychoses:
a make up 1% of psychoses
b are linked to potassium channel antibodies
c are linked to IL-1β
d are linked to calcium channel antibodies
e respond well to antipsychotics.

3 Autism-spectrum disorder is associated with:
a higher than expected brain weight at birth
b lower than expected brain weight by 3 years of age
c increased connectivity between distant areas of brain
d reduced 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 is found compared with controls
b there is reduced microglial activation
c complement C1, C3, C4 protein activity is reduced
d complement C2 protein activity is increased
e the prevalence of C4A allele is increased.

5 Proposed immunotherapeutic approaches do not involve:
a minocycline
b ropinirole
c plasmapheresis
d steroids
e microglial activation *in vitro*.