WHAT IS NEW IN THIS RESEARCH AREA, AND WHAT HAVE WE LEARNED IN THE PAST 18 MONTHS?

In 2011 and 2012, significant progress has been made in understanding the biological causes of ASD, including new observations about differences in neural connections, the discovery of molecular mechanisms that might cause autism symptoms, and insights from some of the disorders that co-occur with autism.

**Neuroimaging findings**

In the past two years there have been over 225 research publications that dealt in some way with neuroimaging of brain structure or connectivity in autism. Important studies have focused on siblings of autistic children, examining neural pathways, and working toward understanding different subtypes of autism. In response to the urgent need for a sensitive and specific biomarker for the diagnosis of ASD, many research groups have been intensely studying patterns of infant development, including prospective longitudinal studies of infant siblings of children with ASD. Some traction has been gained by using diffusion weighted imaging to study white matter pathways. In one report, aberrant development of white matter pathways was found in high-risk infants who later developed autistic symptoms (Wolff et al., 2012). In another, abnormal white matter architecture was found in 3 year old children with autism (Weinstein et al., 2011).

Another trend is to use structural magnetic resonance imaging to define neural phenotypes of autism. Several studies (e.g., Hoeft et al., 2011) have demonstrated that young boys with fragile X have different patterns of brain abnormalities than young boys with idiopathic autism. Other studies have shown that the precocious brain growth associated with autism is observed mainly in young boys with regressive autism (Nordahl et al., 2011) and does not occur in girls with autism. Interestingly, enlarged brain size may be related to ethnicity since macrocephaly is not a common feature of autism in Israel (Davidovitch et al., 2011).

Over the past two years, an array of studies using functional magnetic resonance imaging (fMRI) and diffusion weighted imaging has advanced understanding of the neural circuitry that is affected in ASD. These studies have most often highlighted differences in functional activation within specific brain regions known to be specialized for processing social information (e.g., social orienting, Greene et al., 2011; affective aspects of social processing, Gotts et al., 2012; gaze on emotional faces, Klimann et al., 2012; attention, Redcay et al., 2012). One study described a specific fractionation or disconnection of social brain circuits (Gotts et al., 2012). Diffusion weighted imaging studies found aberrant pathways connecting language areas in children with autism (Lewis et al., 2012).

**Neurophysiological findings**
In a notable study, Elsabbagh and colleagues (2012) demonstrated that investigation at the level of neural circuit function may reveal distinctions inaccessible to behavioral assays alone. EEG responses to dynamic eye gaze shifts during the first year predicted different clinical outcomes at 36 months, despite similar patterns of gaze as measured by eye tracking. In another study these investigators found atypical audiovisual speech integration in infants at risk for autism (Guiraud et al., 2012). As the field strives for earlier methods of detecting autistic development, these remarkable findings, together with the neuroimaging findings described above, offer hope for the possibility of non-invasive, brain-based screening methods that could detect differences prior to the behavioral emergence of ASD.

**Molecular basis and phenotypic autism**

2011 and 2012 saw progress in understanding the biological basis of the monogenic causes of specific ASD syndromes as well as the basis of some of the genetic associations identified in large autism studies. Many of these findings are consistent with dysfunction at the synapse, the point at which neurons communicate with each other. Of particular interest are the insights into the effects of gene mutations in animal models of syndromic autism, including FMRP in fragile X, MecP2 in Rett syndrome and TSC1/2 in tuberous sclerosis. Remarkably, these mouse studies support the hypothesis that many aspects of the phenotype, including the phenotype in adults, are reversible, especially with drugs influencing the mGluR5 receptor or with the mTOR inhibitor rapamycin (Tsai et al., 2012; Silverman et al., 2012; Auerbach et al., 2011). Rare mutations in genes that encode proteins forming large complexes at the synapse (Shank/ProSAP) are associated with autism. Deletions of some of these genes in mice were found to cause autism-like behaviors and alterations of synaptic function and glutamate neurotransmission (Schmiesser et al., 2012). Additionally, genetic deletions or duplications, so called copy number variants, in other genes may interact with mutations in Shank to cause autism (LeBlond et al., 2012).

As genes that confer increased risk for ASD are being identified at an increasing pace (see Question 3), a crucial next step for brain imaging research is to determine how these risk genes impact the development of brain structure and function and contribute to the great heterogeneity observed in people with ASD. One noteworthy fMRI study is among the very first to tackle this important challenge. Rudie and colleagues (2012) demonstrated that a common, functional ASD risk variant in the Met Receptor Tyrosine Kinase (MET) gene is a potent modulator of key social brain circuitry in children and adolescents with and without ASD. MET risk genotype predicted atypical fMRI activation and deactivation patterns to social stimuli (i.e., emotional faces), as well as reduced functional and structural connectivity in temporo-parietal regions known to have high MET expression. If validated, these findings highlight how genetic stratification may lead to an understanding of heterogeneity in ASD and help to elucidate the key changes in neural circuitry.

A study of gene expression in post mortem brains of persons with autism showed a remarkable attenuation of the normal variation between cortical regions found in normal brains, a finding that suggests a simplification of cortical patterning (Voineaugu et al., 2012). The study also uncovered patterns of neuronal gene expression in autism that paralleled and confirmed the role of genes known to be associated with autism risk. Surprisingly, the researchers also found a pattern of immune and glial gene expression in autism brain that has not been seen in the genetic studies, an observation that supports the view that brain immune system responses in autism are likely related to environmental events and not necessarily genetic influences.
New immune findings
Models of microglia and immune pathway function during brain development and neuroplasticity in experimental animals are the strongest demonstration of a role for the immune system in ASD pathogenesis (Schafer et al., 2012; Stephan et al., 2012). The potential role of adaptive immunity, environmental factors, such as maternal infections, and autoimmunity in the pathogenesis of ASD has also been shown in animal studies. Maternal immune activation causes long-term adaptive immune system abnormalities, such as reduction of T regulatory cells and CD4^+ T cells, in the offspring of mice exposed to immune challenges during pregnancy (Hsiao et al., 2012a; Braunschweig et al. 2012). Interestingly, behavioral abnormalities observed in this model were reduced by reversing cellular immune deficits with immunologically normal bone marrow (Hsiao et al., 2012b). The findings in experimental animals are critical for understanding the role of neuroglia and immune pathways in the development of autism and the neuronal circuitry abnormalities.

Although human studies of immune function in ASD have been limited, some observations support a potential link between immune dysfunction and autism. One of those studies evaluated cytokines and chemokines expression in neonatal blood spot samples in the Danish Newborn Screening Bank suggest that a hypoactive immune system was present in neonates that developed autism (Abdallah et al., 2012).

Co-occurring disorders
In 2011-2012 there was increasing recognition of the substantial overlap between ASD and epilepsy. Past studies revealing that 10-20% of individuals with ASD have concurrent epilepsy have been updated by the discovery of rare common roots for ASD and epilepsy: 1) mutations in the gene coding for a metabolic enzyme, branched chain ketoacid dehydrogenase kinase (Novarino et al., 2012); and 2) mutations of the X-linked protocadherin 19 gene (Marini et al., 2012).

Recent studies have also reinforced the overlap between ASD and gastrointestinal disturbances. Mazurek and colleagues (2012) examined 2,973 children with ASD enrolled in the Autism Treatment Network and found that 24% had one or more chronic gastrointestinal problems and that these gastrointestinal problems were associated with higher rates of both anxiety and sensory over-responsiveness.

ASD also overlaps with some types of sleep dysfunction. Children with ASD who slept fewer hours per night had lower overall intelligence, verbal skills, overall adaptive functioning, daily living skills, socialization skills, and motor development. Children who slept fewer hours at night with waking during the night had more communication problems (Taylor et al., 2012). Children with autism spend reduced time in the rapid eye movement (REM) phase of sleep, which has been hypothesized to play a role in neuroplasticity and brain development. The drug donepezil increased REM sleep time in children with autism, but further research is needed to determine whether this impacts cognition, development and behavior (Buckley et al., 2011). Endogenous levels of 6-sulphatoxymelatonin (6-SM), a major metabolite of melatonin (which signals sleep onset), has been documented to be low in adolescents and young adults with autism.
WHAT GAPS HAVE EMERGED IN THE PAST 18 MONTHS?

There continues to be a paucity of studies related to the cellular neuropathology of autism. The dearth of postmortem tissue has slowed research in every area addressed under this question. While this has been a challenge for years, the loss of over 50 brains after a freezer malfunction in June 2012 has been an enormous setback. The loss represented about one third of the largest autism brain repository, and will take years to replace (see Chapter 7).

In order to study the cellular and molecular underpinnings of autism, researchers also need appropriate cell culture models of neurons. In a landmark paper, investigators generated cortical neurons from induced pluripotent stem cells (iPSCs) derived from skin cells of two individuals with Timothy syndrome (Pasca et al., 2011). These neurons showed abnormalities in differentiation and neurotransmitter production that could be reversed by blocking the calcium channel known to be mutated in this monogenic cause of autism. iPSCs are promising both as a biological tool to uncover the pathophysiology of disease by creating relevant cell models and as a source of stem cells for cell-based therapeutic applications and drug discovery. It is noteworthy that DeRosa and colleagues (2012) recently described, for the first time, the derivation of iPSC lines from the whole blood of children with ASD.

The lack of longitudinal studies remains a striking gap in studies of brain function in ASD. While cross-sectional studies have provided important findings, there is a dearth of essential information about the time course of brain development from early infancy to adulthood (and, the yet to be studied aging brain) in ASD. The power of longitudinal studies was recently reinforced by the results of a longitudinal structural MRI study that identified an increased rate of amygdala growth in very young children with ASD (Nordahl et al., 2012). These researchers demonstrated that the amygdala is larger in children across ages 2 and 4 years; however, the rate of amygdala growth during a 1-year interval was faster in children with ASD than in typically developing children, even after controlling for the rate of whole brain development.

Though a network has been launched to study ASD in females, there remains a pressing need to conduct research aimed at understanding all aspects of ASD (genes, brain, and behavior) in females with ASD. Because ASD disproportionately affects males, the skewed sex ratio has resulted in a bias of published research towards studies of males. Dworzynski and colleagues (2012) found that girls are much less likely to be diagnosed with autism than are boys, unless they also have intellectual or behavioral problems. The authors conclude that this might reflect gender bias in diagnosis or genuinely better adaptation/compensation in girls.

A new gap area is to understand the role of the immune system and microglia in autism, which have recently been found to contribute to brain development and plasticity (Stephan et al., 2012).
Another is to understand the generalizability and pathophysiological significance of the findings of increased oxidative stress markers in plasma of children with autism (Melnyk et al., 2011).

Word count: 2045

References:


