Central Role of Voltage Gated Calcium Channels and Intercellular Calcium Homeostasis in Autism

(by N.B.S. Lozac, first version published online February 2007, last updated April 2010)

**part 2:**

**Dysregulating Factors:**

- **Genetic Factors** – page 2
- **Hypoxia/Ischemia** – page 4
- **Toxins** – page 6
- **Infectious Agents** – page 9
- **Maternal Factors** – page 19
- **Thyroid Function** – page 21

**Conclusion** – page 22

Appendix i: **HIV and autism** – page 23

Appendix ii: **Summary of Abnormal Biomedical Findings in Autism** – page 30
**Genetic factors**

There are presently two identified genetically inherited disorders of mutations in calcium channels with high incidence of autism. Timothy syndrome is caused by mutations in CACNA1C, the gene encoding Ca(V)1.2 calcium channel, causing loss of channel inactivation and suspected intracellular calcium overload. This dysfunction causes a multisystem disorder including congenital heart disease, webbing of fingers and toes, immune deficiency, intermittent hypoglycemia and cognitive abnormalities. Frequent seizures, irregular sleep patterns, dysmorphic facial features, myopia, motor abnormalities and small and decaying teeth have also been recorded in affected individuals [15454078]. The exact incidence of autism is unknown but could be as high as 80 percent.

A mutation in a calcium channel gene CACNA1F, encoding for Cav1.4 L-type calcium channel, that results in retinal disorder and visual impairments has been observed in a New Zealand family. Although female members of the family display visual impairments, the symptoms are more severe in male family members. Five of the affected males exhibit intellectual disability, with autism being present in three of those five individuals [15807819]. (see Motor/Sensory and Gender).

A study looking at mutations in genes encoding for calcium channels in a broader sample of 461 individuals with autism found that 6 of them expressed a CACNA1H mutation, relating to Cav3.2 T-type calcium channel. Although it was clear that the identified mutation was not solely responsible for the condition, as some of the nonaffected family members were found to carry the same mutation, it was suggested that it could contribute to the development of the ASD phenotype or influence its expression [16754686]. CACNA1H chromosomal location 16p13.3 is shared with with tuberous sclerosis 2 gene (see below).

Similarly, a mutation in the gene encoding for Shank3, a scaffolding protein that forms signalling complexes with at least two of the voltage gated calcium channels, was recently identified on chromosome 22q13 in a small number of affected individuals [awaiting publication]. Shank proteins are involved in calcium-mediated activation of gene transcription factor CREB. (see Brain). The haploinsufficiency of Shank3 is thought to be the responsible for the neurological deficits of 22q13 Deletion Syndrome, in which an estimated 50 percent of affected individuals exhibit autism-like symptoms [15286229]. Of note in this context that chromosome 22q13 has been identified as one of the preferred location for integration of human herpesvirus 6 DNA [9753061] (see Viruses).

Calmodulin is another cellular protein that binds to LTCC in neurons and other excitable cells and plays an important role in regulating their activities and signalling in CREB activation pathway [11598293, 16685765]. Calmodulin binds to tuberin, and its binding site is altered by mutations linked to tuberous sclerosis [11811958, 17114346]. Tuberin is encoded by tuberous sclerosis 2 gene, located on chromosome 16p13.3 (see above). Mutations in tuberous sclerosis genes 1 and 2 lead to a rare genetic disorder characterized by seizures, mental retardation, skin lesions and impaired functioning of many organs, including brain, kidneys, heart, eyes and lungs. About half of affected individuals have learning difficulties and a reported 16% meet the diagnostic criteria for autism [16901420].

22q11.2 Deletion syndrome (DiGeorge/velocardiofacial syndrome) is characterized by congenital cardiovascular disease, dysfunction of parathyroid gland, immunodeficiency, neurodevelopmental and psychiatric disorders. Autism spectrum disorders as well as related Attention Deficit Hyperactivity Disorder and sometimes Obsessive Compulsive Disorder are common in children with the syndrome [16926618]. Affected children show hypoparathyroidism and abnormal calcium homeostasis and, because of depressed cell-mediated immunity, serious bacterial, viral and fungal infections [6973633, 16995575]. Behavioral manifestations of this syndrome has been hypothesised to result in part from haploinsufficiency of the catechol-O-methyltransferase (COMT) gene, located within the 22q11 region [10643919].
Individuals with Cowden Syndrome, caused by mutations in PTEN tumour suppressor gene, sometimes exhibit neurobehavioural symptoms similar to autism. Germline mutations in the gene have been identified in a small subset of individuals with non-syndromatic autism, as well as some non-affected family members [15805158, 11496368]. It has been suggested that inactivation of PTEN leads to behavioral abnormalities seen in this disorder. It is of interest that inactivation of PTEN has been observed to result in enhancement of LTCC current in cardiac tissue [16627784].

Autism often co-occurs with phenylketonuria (PKU), a genetic disorder in which the body lacks phenylalanine hydroxylase, the enzyme necessary to metabolize phenylalanine to tyrosine. A recent study has found that amongst the genes upregulated by phenylalanine, and thus likely to be affected in PKU disease, were L-type calcium channels, calcium/calmodulin-dependent protein kinase (CaMK II), several genes related to transmitter release, some glutamate receptor subunits and glutamate transporters [15127128].

(Also see Membrane for Smith-Lemli-Opitz Syndrome).

Several polymorphisms in the genes encoding proteins whose activity is directly modulated by calcium have been suggested to play a possible role to autism [17275285]. Although no firm genetic linkage has been established, it has also been hypothesised that mutations in genes encoding sodium channels SCN1A and SCN2A, and those encoding potassium channels, such as CASPR2, may play a role [12610651, 10673544]. On the other hand, physical disruption in the gene KCNMA1 encoding BKCa channels and decreased activity of these channels in autism have been observed recently [16946189]. Recent findings have pointed to functional coupling of BKCa and LTCC channels on plasma membrane, and in addition excessive calcium levels as result from either extracellular space or intracellular store-released, are known to modulate functioning of potassium channels [15141163, 16828974, 15486093] (see also Epilepsy). It may be worth noting that KCNMA1 gene location on chromosome 10q22 has been implicated in preeclampsia [15208369] and also that this chromosomal location has been identified as a preferred integration site for hepatitis B virus [9519839].

Equally important are the findings of increased frequency of polymorphisms in the genes related to the immune system in autism, possibly indicating weakened defenses against and elimination of pathogens such as viruses and bacteria from the body, leaving a developing nervous system especially vulnerable to direct and indirect pathogenic influences (see Immune and Viruses). In addition to several mutations in genes belonging to major histocompatibility complex (MHC) region, related to immune function, being associated with autism, the involvement of CREB-mediated events in regulation and expression of these genes should be also of interest [16730065] (see above, also see Brain).

One very interesting finding in recent times was the association of genetic polymorphisms related to macrophage migration inhibitory factor (MIF) in individuals with autism [18676531]. MIF is central in host immune reactions/viral clearance and inflammatory responses. MIF favours viral neuroinvasion by compromising the integrity of the blood-brain barrier (see Infectious Agents re viral/bacterial aetiology in autism). It is very closely linked to MCP-1 (elevated manifold in autism) and other proinflammatory chemokines/cytokines, and its levels are inversely related to regulatory cytokine IL-10 (low in autism). MIF also plays a central part in gastrointestinal inflammation (see Gastrointestinal), as well as cellular oxidative stress pathways - cysteine mediated redox mechanisms (impaired in autism, see Oxidative Stress). It is also appears to be directly involved in neuronal function via at least one pathway, that of Angiotensin II. Levels of MIF are often suppressed in fever. The expression levels of MIF gene are partly regulated by calcium-dependent CREB.

In addition, autism has been linked to some genetic mutations and polymorphisms that raise susceptibility to oxidative stress (see Oxidative Stress).
Oxygen and glucose deprivation in autism and role of calcium signalling

Neonatal hypoxia has been hypothesised to play an etiological role in development of autism. In animal models, hypoxia and hypoxic brain lesions are associated with some of autism-related neurobehavioural symptoms, including withdrawal in social and novel situations, diminished exploration in novel fields and repetitive behaviours [14985924, 16482712, 8075818].

Both acute and chronic oxygen and glucose deprivation lead to changes in membrane proteins, include excitation of chemoreceptors as well as vasoconstriction and systemic vasodilatation (see Cerebral_Blood_Flow). Hypoxia inhibits several potassium channels, leading to membrane depolarization and excessive calcium entry through LTCC, and subsequent downstream effects including release of calcium from intracellular pools, contributing further to cytosolic calcium overload. These events are thought to be G-protein linked [16828723]. Although a variety of membrane ion channels seem to be affected by hypoxic conditions, LTCC seem to be most significantly affected [14643931].

Neuronal damage during cerebral ischemia is thought to be mediated mainly through increases in intracellular calcium. The activation of LTCC and subsequently ryanodine-sensitive intracellular stores during oxygen and glucose deprivation plays a major role in these effects, which can be reduced in vitro by several calcium blocking agents [9445351, 10826535]. In addition, in several animal studies application of nimodipine resulted in prevention or reduction of adverse behavioural and neurochemical effects of perinatal hypoxia. In one study it was observed that nimodipine also enhanced the early postnatal development of calcium-binding proteins [8817697], while another study showed that alongside reducing negative behavioural consequences, nimodipine reduced adrenal dysfunction and abnormal corticosterone stress response in rats [8075818] (see Hormones). A study looking at energy metabolism in the developing brain damaged by aglycemic hypoxia found that calcium influx through LTCC mediated these effects. Application of diltiazem or verapamil but not nifedipine significantly improved the recovery from aglycemic hypoxia, manifested in decreased ATP energy state and increases in lactate [8897472] (see Mitochondria). Nimodipine and verapamil normalized cerebral pH following middle cerebral artery occlusion in another rodent model of ischemia [3793803]. It has been suggested that the dual blockade of calcium entry using MK-801, a NMDA antagonist, alongside nimodipine may be a useful tool for protection against ischemic brain damage in clinical practice [1985301].

Upregulation of expression of several chemokine receptors of CNS and their activation has been observed following perinatal hypoxia and ischemia in animal models [16516309, 15467356] and absence of the chemokine receptor CCR2 protects mice against cerebral ischemia/reperfusion injury [17332467]. Both neuronal and glial cells possess a variety of chemokine receptors that can regulate calcium and other signaling pathways - in normal circumstances chemokine modulation of calcium homeostasis is believed to have positive effects on neuronal development, but excessive activation and expression of these receptors could have long term effects on their function and gene expression, as well as raise cellular vulnerability to viral and other proteins that act as chemokine receptor agonists (see Viruses). One of the pathways activated by chemokines is the calcium-mediated CREB (see Brain) [11958818].

It is worth noting in this context that hypoxia is thought to have a negative effect on the immune system – in addition to its modulating effects on various genes related to immune and inflammatory function observed in vitro [16849508], in vivo studies have further confirmed these findings – one example is the increased risk of infection by dysregulation of Th1/Th2 cytokine balance and decrease in T lymphocyte numbers, as recorded in volunteers exposed to high altitudes [15870630]. On the other hand, the opposite mechanism has also been proposed, whereas fetal exposure to infection (see Viruses) and pro-inflammatory cytokines
may reduce the threshold at which hypoxia becomes neurotoxic, and so make the brain more vulnerable to hypoxic insults [15707712].

It has been proposed that impairments of motor coordination following hypoxia might be the result of altered function of Purkinje neurons [16169666] (see Brain and Motor/Sensory). Perinatal anoxia has been observed to have negative effect on development and functioning of auditory systems, also closely linked to LTCC function [16365292] (see Auditory).

Periods of prolonged hypoxia are associated clinically with an increased incidence of dementia and raised susceptibility to development of neurological disorders like Alzheimer’s disease. It has been suggested recently that hypoxic channel up-regulation is dependent upon formation of amyloid beta peptides, which induce excessive activation of LTCC [16464656, 16321794, 12392105]. Blocking calcium channels by several antagonists has been shown to be neuroprotective in models of Alzheimer’s disease (see Related Disorders).

Amongst other types of cells the effect of hypoxia-induced dysregulations in calcium traffic through voltage gated channels in arteries may be of most relevance. A study looking at effect of hypoxia on lateral artery in striatum, a subcortical area of the brain, found substantial gender difference in its vulnerability to hypoxia. Chemically induced hypoxia in rats lead to calcium overload and cell death, lesions, edema and immune activation. These effects and subsequent motor disturbances were highly sex-dependent and modulated by changes in hormonal levels, with males being much more susceptible than females (see Gender_Differences) [9678634].

In gastrointestinal tract, mucosal hypoxia is closely associated with chronic inflammation, and these events are dependent on alterations in the expression and function of CREB, whose phosphorylation is in great part regulated by calcium flux through LTCC [15253703] (see Gastrointestinal and Brain).

Lastly, LTCC are thought to mediate hypoxia-induced vasoconstrictive responses in human placenta, which could be completely abolished by a relatively low doses of nifedipine [16368136].
**Toxic Agents**

Environmental factors have long been associated with a wide range of neurodevelopmental deficits. At high levels of prenatal exposure environmental toxins are capable of producing cognitive, motor, behavioural and sensory disturbances, whereas the effects of lower levels of exposure on the fetus are suposedly more subtle and dependent on the timing and dose of the chemical agent. For example perinatal and childhood exposure to high doses of heavy metals lead and mercury can result in encephalopathy, convulsions or cerebral palsy. The effects of lower-dose exposures are less clear, although behavioural alterations and impairments in intellectual function and attention, as well as increased seizure susceptibility have been suggested [16136561, 12224761, 15970329, 2294437]. Recent animal research points to influence of perinatal exposure to various toxins on neuronal development and functioning. It has been proposed that exposure to PCBs as well as various other synthetic chemicals may contribute to common developmental disorders [12060832, 12060832, 17268844].

Ion channels are a target of a many environmental toxins that are capable of altering their activity. Numerous environmental agents, including low frequency electromagnetic fields, affect functioning of membrane as well as intracellular calcium channels, thus affecting cellular calcium homeostasis, in many types of cells, including neurons. These effects are widely believed to the main mechanism behind their neurotoxicity [15000519, 8247389].

Of special interest might be the findings of a series of murine studies pointing to links between viral infections and absorption and tissue/organ distribution of environmental toxins, in the light of reports indicating impaired detoxification and accumulation in the body of environmental toxins in ASD individuals (see Infectious Agents).

*Lead*

While lead competes with calcium at the plasma membrane for entry into the cell, it also interferes with intracellular calcium buffering systems and its regulatory actions in various cell functions [8247414, 7504228]. Through these actions lead is believed to be able to disrupt the blood-brain barrier, both by damaging the astrocytes and the endothelial cells [1671748, 9622620] (see BBB). Developing nervous system is thought to be particularly sensitive to these effects, as lead is capable of rapidly crossing the blood brain barrier and affecting neuronal function, and long-term effects of lead poisoning are believed to result in various cognitive, behavioural and motor impairments [16501435, 12835126, 11106867].

While several types of ion channels are affected by lead, L-type calcium channels appear particularly sensitive. It is of interest to note that, while the main action of lead is the blockade of these channels and inhibition of calcium traffic, several in vitro studies have also noticed some unexpected effects, like the enhancement of calcium flow in some cells or the sudden unblock of channels following strong depolarisation [1660583, 9223552].

*Iron*

Although iron is an essential mineral for normal cellular physiology, its excessive levels can result in damage to the cell. Impaired calcium homeostasis and oxidative stress with excessive production of ROS (see Oxidative Stress) is believed to be one of the consequences of both acute and chronic body iron overload.

Various calcium channel antagonist have been observed in vitro and in animal studies to attenuate the neurotoxic effects of iron by inhibiting excessive entry of both iron and calcium ions, implicating effects of iron on membrane permeability and permissiveness of calcium channels [16528447, 12184662, 12937413]. In addition to overactivation of voltage gated channels, iron toxicity also appears to be closely related to excessive activation of NMDA glutamate receptors [16908409].
Mercury

Both organic and inorganic mercurials are neurotoxic. The neurotoxic effects of both acute poisoning and long-term environmental exposure to mercury toxins are attributed in great part to their interaction with calcium channels and modulation of levels of calcium in the cell [12062935, 16326920, 16465247, 7641225].

Activation of M3 muscarinic receptors was found to contribute to elevations in intracellular calcium and by methylmercury. Prior down-regulation of muscarinic and IP3 receptors with Bethanechol protected against neuronal cell death, indicating interaction of mercury with muscarinic and IP3 receptors to cause calcium release from the intracellular pools [15141107]. Of note in this context are the reported benefits of Bethanechol treatment in a subgroup of individuals with autism [10867750].

Migration of neuronal and nonneuronal cells during development was observed in vitro to be strongly influenced by calcium oscillation and sensitive to dose-dependent thimerosal-induced releases from intercellular stores [16720042]. This increase of responsiveness of IP3 receptors by thimerosal, an organic mercury compound, appears to be able to modulate sensitivity to calcium of parathyroid cells [10598273].

In addition to effects of mercury on neuronal cells, of equal interest are the findings of its negative effects in immune responses. Disturbances of calcium homeostasis induced by low-level chronic exposure to mercury is suggested to be one of the mechanisms behind immune dysfunction and tendency towards development of chronic disease following viral infection [9653674, 8952707]. Both organic and inorganic mercury have been observed to screw immune responses and induce autoimmunity in mice, with mouse strains that are genetically susceptible to autoimmunity showing most pronounced developmental disturbances following exposure to ethylmercury [17084957, 15184908] (see also Viruses/Bacteria). Dendritic cells, crucial for the first-line response of the immune system towards external pathogens, have recently demonstrated particular sensitivity to changes in calcium levels as induced by ethylmercury-containing preservative thimerosal [16835063] (see Immunity/Inflammation).

The tendency of increased persistence of virus with methylmercury exposure may turn out to be of particular relevance in autism, as this toxin has been observed in a murine model to change viral myocarditis in a direction compatible with the development of chronic inflammatory disease. Amongst other markers significantly raised levels of calcium and decreased levels of zinc were recorded in the inflamed heart [11314973, 8682094].

The continuous application of heavy metals lead and mercury in vivo resulted in their accumulation in brain cells and the occurrence of delayed toxic effects in rat fetuses. It was shown that when methylmercury is applied at non-toxic concentrations it becomes neurotoxic under pro-oxidative conditions. Lead and mercury induce glial cell reactivity, a hallmark of brain inflammation and increase the expression of the amyloid precursor protein. Mercury also stimulates the formation of insoluble beta-amyloid, which plays a crucial role in the pathogenesis of AD and causes oxidative stress and neurotoxicity in vitro. Based on their results and reviews of previous findings authors suggest that those heavy metals may contribute to the etiology of neurodegenerative diseases [16898674] (see Related Disorders).

Other toxic agents

Various other environmental toxins are capable of modulating the functioning of calcium channels, including various organophosphates, phthalates and other industrial compounds used as pesticides, solvents, flame retardants, plasticisers etc [11750043, 16300829, 16686424, 16325978, 10869474]. Nerve agent Sarin and structurally similar organophosphates have demonstrated ability to induce changes in neuronal gene expression, with calcium channel proteins being some of the most affected ones [16376859]. Of particular interest are the observations of heightened vulnerability to some of these agents due to inborn
weakness in detoxification pathways in some individuals and their association with autism [16027737, 17084875].

Carbon monoxide [11743890], sulfur dioxide [16857312], cadmium [15954739], nicotine [15764844], ethanol [16555300], low frequency electromagnetic fields [17941084, 15036948 15110298], ultrasound [2685832] and X-rays [9096258] are amongst some of the other factors capable of modulating membrane permeability and cellular calcium metabolism.

In vitro and animal studies have demonstrated that various calcium antagonists, in particular nimodipine and other dihydropyridines, are able to attenuate negative effects of these agents on cellular function and survival [17941084, 16635102, 2156356].
Infectious agents as disruptors of calcium homeostasis and possible implications for autism

One proposed etiology for autism is viral infection very early in development. Various viruses and other infectious pathogens have been implicated as etiological factors, including several members of herpes family, rubella, influenza, measles and mumps viruses. In addition, individual cases of prenatal syphilis and toxoplasmosis each have been reported in literature [15804954, 222139, 4947755].

The best association to date have been made between perinatal cytomegalovirus and rubella viruses and autism. Although detailed large scale follow-up studies have yet to be carried out, numerous case reports describe perinatal cytomegalovirus infection in association with development of autism [12959425, 6315673, 6086566]. It is estimated that up to 15% of asymptomatic congenital infections by CMV in the neonatal period develop persistent problems, which often involve neurological disorders and which typically appear after a period of time, thus making diagnosis difficult or impossible [15973639]. With regards to rubella virus, one follow-up report on psychiatric and behavioral consequences of documented congenital infection recorded significantly high rates of mental retardation and behavioural pathology, including autism [702254].

Several case reports in literature describe late-onset of autism following herpes encephalitis [12369775, 3558293, 1743418].

A recent study found polyomavirus infection in postmortem autistic brains BKV, JCV, and SV40 viruses were significantly more frequent among autistic patients compared to controls [20345322].

Also worth noting is the finding that a large subset of subjects with autism shows evidence of bacterial and/or viral infections not present in age-matched controls [17265454], as well as a reduced response to vaccine antigens, especially Bordetella pertussis [link]. In addition, there appears to be a correlation between virus serology and brain autoantibodies in autism (see Immunity/Inflammation).

Viruses

Animal models in which early viral infection results in behavioral changes later in life include the influenza virus model in pregnant mice and the Borna disease virus model in newborn Lewis rats. Neonatal Borna disease virus (BDV) infection of the rat brain is thought to be a useful model for studying the pathogenesis of neurodevelopmental abnormalities in humans, as it produces neurodevelopmental damage in rodents that is similar to some pathological and clinical features of autism and schizophrenia. Amongst other things neonatal BDV infection profoundly affects social behaviors in adult rats [16860408]. Another significant finding is that the same virus was found to produce differential neuroanatomical and behavioral abnormalities in genetically different inbred rat strains [12106670].

Of note is that neonatal rats infected with BDV do not mount an aggressive response to the virus like their adult counterparts, but instead develop a persistent infection with less obvious clinical symptoms. Postmortem examinations of the rats’ brains reveal significant loss of Purkinje and dentate gyrus granule cells, as well as degeneration of the parts of hippocampus [10321982]. Similarly, selective neuronal damage was observed in BDV replication in hippocampal slice cultures from rats, where damage and death of granular cells was accompanied by reduced density of mossy fiber axons [16140749].

BDV infection in rats is also associated with elevated expression of cytokine and chemokine mRNA, as well as abnormal levels of neurotransmitters, including increased tissue concentration of serotonin in parts of brain [15385249]. Significant abnormalities in brain
levels of metallothioneins and zinc metabolism have also been observed, leading to suggestions that such disturbances may contribute to neurodevelopmental damage in perinatal viral infection [16612977] (see below).

Prenatal influenza viral infection in mice leads to development of abnormal emotional and cognitive functions and behaviours, including deficits in social interaction, in adult offspring [12064515, 12514227]. The infection causes differential expression of genes in brains of the infected mice [15906383] as well as atrophy of pyramidal neurons and increased brain size in adulthood [12064515]. These findings were confirmed by another study, where it was noted that the alterations in gene expression became apparent only after a latency period, an observation which may be of significance in autism [15973158].

Upregulated expression of glial fibrillary acidic protein (GFAP), an important marker of gliosis, neuron migration, and reactive injury, has been recorded [12140787]. Of note is that significant elevations in levels of GFAP, as well as autoantibodies to the protein, have been observed in autism through plasma, cerebrospinal fluid and postmortem brain examinations [16147953, 9308986, 8353169]. In addition, reduced levels of Reelin have been observed in mouse offspring, implicating the involvement of this protein in neuronal migration abnormalities in mouse brains following viral infection [10208446] (see Brain re Reelin abnormalities in autism).

Developmental abnormalities in paediatric Human Immunodeficiency Virus (HIV) infections are of great relevance for establishing mechanisms that lead to autism. Children that are pre- or postnatally infected with the HIV subsequently developed neurological symptoms, including impairments or declines in cognitive, language, socio-emotional and motor development. In some cases regression/deterioration of previously acquired skills can happen several years after the original infection, the longest reported being in a child of 5 years of age, who previously exhibited age-appropriate behaviours and development of all areas of concern [7682171, 7770287, 8935240]. Most importantly, improvements in neurological disturbances are often noted following antiviral or immune-modulation therapies. These improvements include diminishing of autistic symptoms and recovery, sometimes complete, of language and cognitive abilities, normalised play and social behaviour, and improvements in motor skills, muscle tone and visual-spatial functions [2493377, 9493492] further reading: [link]

The central mechanism behind the neurodevelopmental decline and behavioural problems in paediatric HIV infection is thought to be the neuronal injury caused by excessive increases of intracellular calcium induced by HIV viral proteins [16553776, 7847672]. In addition to HIV, numerous other viruses, including many that have been implicated in autism, code for proteins that are capable of inducing similar perturbances in calcium homeostasis in neurons and other types of cells. Calcium plays an important role in replication cycles and pathogenesis of many viral diseases and viruses have been proposed to influence calcium homeostasis of host cells through several different pathways.

An estimated third of the adults and half of the children with acquired immunodeficiency syndrome (AIDS) induced by HIV exhibit neurological disturbances. This is usually referred to as AIDS-induced dementia, neuro-AIDS or HIV encephalitis, and the symptoms include language and attention problems, lethargy, motor and sensory dysfunction and abnormal reflexes. Affected children exhibit many autistic symptoms, including language and socio-behavioral abnormalities (see above). Some of the pathologies reported in the brain of patients with AIDS are neuronal injury and loss, including myelin loss and axonal damage, and microglial activation.

Dysregulation of calcium homeostasis underlies neurological disturbances in neuro-AIDS. The virus seems to enter the brain in the early stages of infection and arising pathological processes in the brain and CNS are only in part affected by HIV-dependent processes in the periphery. Although the virus does not seem to directly infect the neurons, two of its identified viral proteins are neuroxic - the viral coat protein gp120 and the transcription regulator Tat. Both of these proteins can induce apoptosis of cultured neurons and can render neurons
vulnerable to **excitotoxicity and oxidative stress**. Both gp120 and Tat disrupt neuronal calcium homeostasis by perturbing calcium-regulating systems in the plasma membrane and endoplasmic reticulum. By altering voltage-dependent calcium channels, glutamate receptor channels, and membrane transporters, these proteins promote excessive calcium entry, production of free radicals and mitochondrial dysfunction [12394783] (see Oxidative_Stress and Mitochondrial issues in autism). These effects are at least partly mediated via their effect on chemokine receptors, which are widely expressed on neurons and some of which are able to activate the calcium and cAMP-dependent transcription factor CREB (see Brain) [9826729]. In addition, glial cells were also found to contribute to chemokine upregulation in the CNS and so to contribute to neurological damage [15163738, 11744246]. (see chapter below re chemokine receptors on neurons and glial cells).

Of particular relevance to autism could be the observation of the **ability of retroviruses to cause neurological damage in the absence of lymphocyte infiltration, primarily through upregulation of chemokine MCP-1** [15163738].

Decreased plasma ratio of tryptophan observed in HIV infected subjects has also been suggested to have possible implications for development of neuro-AIDS. Low levels of tryptophan have been proposed to cause a decrease in serotonin synthesis in the brain, which is believed to impair functioning of some types of neurons [9026369]. Brain serotonin level is suspected to play an important role in developing brain and impairments of serotonin metabolism have been implicated in autism. Reduced brain levels of tryptophan and/or serotonin and its receptors have been recorded in other viral infections. Several in vitro studies have noted the inhibitory effects of serotonin and its receptors on the function of VGCC and neuronal migration during development [11976386] (see also Brain and Maternal_Factors).

Various calcium antagonists, acting on both voltage channels and glutamate operated NMDA receptors, are effective in vitro in reducing neuronal damage induced by HIV proteins. Reductions in glutamate levels also protected neurons from gp120-induced injury, suggesting that they act synergistically and that both are necessary for neuronal cell death [1656845]. Calcium channel blocker nimodipine has shown promise for HIV-induced neurological disorders in a Phase I/II clinical trial [9674806].

In addition to HIV, Maedi Visna virus, another lentil virus, often causes a variety of neurological symptoms in infected sheep. This effect once again is thought to be mediated via neurotoxic effects of its Tat protein and could be attenuated in vitro by application of calcium channel antagonists [8552302].

The effect of HIV proteins on chemokine receptors and modulation of calcium fluxes in immune cells is thought to play an important role in HIV-induced immune dysfunction (see Immune/Inflammation). Similar mechanisms are thought to lie behind HIV disturbances of gastrointestinal function (see Gastrointestinal).

Several viruses from the herpes family have been implicated in the etiology of autism (see above). Some of the herpesviruses are neurotropic and are involved in the pathogenesis of neurological symptoms in infants. Cytomegalovirus (CMV) infection is the most frequent congenital infection in humans and can cause permanent damage, often following initial asymptomatic period. The most frequent symptoms are mental retardation, hearing loss, microcephaly as well as hydrocephalus, periventricular calcification, neuromuscular disorders and chorioretinitis or optic atrophy [6159568].

In vitro CMV was found to prevent induction of differentiation of human neural precursor cells into neurons. The virus arrested cell growth and induced apoptosis in infected cell cultures and this effect of CMV was proposed as a possible explanation for the abnormalities in brain development associated with congenital infection [16940505]. A study looking at intracellular calcium responses to CMV infection noted excessive influx of calcium shortly after the infection of cultured human fibroblasts, with substantial involvement of intracellular stores in addition to voltage gated plasma channels. Application of calcium
channel blockers inhibited the rise in intracellular calcium levels. Authors of the study suggested that the observed effects of the virus on calcium metabolism may be related not only to the development of CMV pathology, but also to viral replication itself, since in other studies cyclic nucleotide modulators and calcium influx blockers were found to inhibit the replication of CMV [3029971].

CMV-induced rise of intracellular calcium is thought to be behind the ability of this virus to interfere in cellular differentiation pathways, including inhibition of macrophage differentiation, thus contributing to CMV immunosuppressive effects [15470031].

Human herpesvirus 6 (HHV-6) encodes proteins that function as highly effective and adaptive chemokine receptor agonists, attracting cells that express those receptors. HHV-6 encoded U83A gene is able to induce significant calcium mobilization in cells expressing chemokine receptors CCR1, CCR4, CCR6, or CCR8. It has been proposed that this newly uncovered mechanism could have wider implications for neuroinflammatory diseases [16332987, 16365449] (see also Gastrointestinal and Immunity/Inflammation re herpesvirus viral proteins, chemokine receptors and calcium homoestasis in immune cells, )

Significant abnormalities in calcium metabolism of influenza virus-infected cultured cells are brought on by enhancement of permeability of membrane calcium channels and also by mobilizing calcium from intercellular stores [3135328, 6626708]. Similar to CMV, influenza viral replication could be inhibited in vitro by a calcium channel blocker, as well as by a drug which binds to calmodulin and so inhibits calcium/calmodulin intracellular pathways that are necessary for virus assembly [6743023, 6808973]. One of the proteins encoded by coxsackie virus, protein 2B, increases membrane permeability and release of calcium stored in endoplasmic reticulum, and in this way disrupts cellular calcium homeostasis, eventually causing membrane lesions that allow release of virus progeny [9218794, 12903773]. Similar effects have been noted following infection of cultured human fibroblasts by poliovirus, another enterovirus from Picornavirus family [7609085]. (see also Immune/Inflammation)

Coxsackievirus B3, a strain capable of inducing myocarditis, has been observed to increase calcium inflow through LTCC in cardiomyocytes, an affect which could be inhibited in vitro by application of calcium antagonist taurine [10375733]. Calcium blocker verapamil had similar effects in a murine model of virally-induced inflammation of heart muscle. Pretreatment with the drug before and during the infection, as well as administration of the drug several days after the infection significantly reduced the microvascular changes and myocardial necrosis, fibrosis, and calcification leading to cardiomyopathy [1331179].

Of particular relevance to autism could be the observation concerning the role of autoantibodies to beta adrenoceptors, since these g-protein linked receptors are expressed in the brain as well as the heart and their activation is linked to cAMP and membrane calcium channels. A study looking at the effects of autoantibodies against beta(1)-adrenoceptor in hepatitis virus myocarditis found that they were capable of inducing arrhythmias and/or impairment of heart muscle, an effect that is most likely mediated by LTCC [15069720].

In a similar manner cardiac abnormalities expressed in congenital heart block in newborns born to mothers with autoimmune disease are caused by maternal autoantibody-mediated disturbance of LTCC function [11257091] (see Maternal_Factors).

Mouse hepatitis virus, a murine coronavirus, induced rapid and transient increases in intercellular calcium via LTCC in a small percentage of cells infected in vitro. It was concluded that the cells that responded with enhanced calcium signals were those that had been infected with multiple viruses and undergone rapid viral replication. Furthermore, several calcium channel blockers and the calcium chelator EGTA inhibited virus infection in this model [9417866]. Several viral proteins Human hepatitis C virus, appear to contribute to reactive oxygen species (ROS) generation by mechanisms that involve dysregulated calcium metabolism, including calcium uptake by mitochondria [16958669] (see also Mitochondria).
Mumps virus, which causes a persistent non-lytic infection, was shown to alter the function of membrane and intercellular calcium channels in cultured neuronal and glial cells in several studies. It has been suggested that this occurrence may reflect a disturbed glia-nerve cell interactions [1647243, 1647243]. Although a reduction in the influx of calcium ions during early stages of infection by mumps virus was recorded, neuronal degradation was almost completely inhibited by nifedipine in one study and by dantrolene, a drug which inhibits release of calcium from intracellular stores, in another [1662994, 9372458].

Apart from its better-known immunosuppressive role, it is believed that in rare cases measles virus may cause progressive neurodegenerative disease [14527283]. Inhibitory effect of the calcium antagonist, verapamil, on measles viral replication in cultured cells has been observed [8454440]. However another study concluded that this effect was probably not related to verapamil block of extracellular calcium entry. It is suggested that, similar to the abovementioned effects of calcium modulators on influenza virus, this is due to drug interference in the calcium/calcmodulin intracellular pathways that are necessary for virus assembly [16054245]. On the other hand the immunosuppressive effect of measles virus likely caused by one of its protein binding to nucleoprotein receptor on cell surface, which triggers sustained calcium influx and inhibits spontaneous cell proliferation [14557619].

Rotavirus tends to effect gastrointestinal epithelial cells, causing acute gastroenteritis and occasionally lactose intolerance (see Gastrointestinal). Growing line of evidence in recent years is pointing to the ability of the virus to leave gastrointestinal tract and cause a broad range of systemic diseases in a number of different organs, including the CNS. Several case studies describe patients who developed seizures and other neurological problems following rotavirus infection. In one particular case diffuse inflammation of the brain was detected by MRI. Neurological symptoms in the patient included screaming fits and seizures, loss of language and social interaction, and reduction in muscle tone. These symptoms persisted in long after the disappearance of gastrointestinal problems [11731961, 12454200].

Animal studies detected replicating rotavirus in infected mice in, amongst other places, macrophages in the lungs and blood vessels, indicating a possible mechanism of its dissemination throughout the body. Interestingly, viral spreading and replication was noted even in the absence of diarrhea. Of note is that in the animals that were infected past a certain stage of development the virus seemed not to infect the brain, as was the case in younger animals. It was concluded that dissemination of rotavirus to the brain in mice is age restricted [16641274].

Rotavirus entry, activation of transcription, morphogenesis, cell lysis, particle release, and the distant action of viral proteins are calcium dependent processes. Viral protein synthesis modifies calcium homeostasis, and rotavirus infection induces increases in intracellular calcium concentration, most likely due to its protein NSP4-induced increases in membrane permeability and calcium influx. Application of LTCC antagonist was shown to reduce the amount of calcium entering the cells, thus suggesting that rotavirus infection of cultured cells activates and/or modulates a specific channel rather than forming a nonspecific ‘leak’ in the plasma membrane [11020376, 9971833].

The observation of the interaction of rotavirus protein NSP4 with membrane protein caveolin-1 during viral infection [16501093] could prove to be of some significance in autism and/or epilepsy, since caveolin-1 is expressed by neuronal cells and seems to exert significant effect on functioning on several ion channels, including VGCC, and on cellular excitability [16040758] (see Membrane_Metabolism). In addition, caveolin-1 seems to play an important role in some inflammatory pathways and in the maintenance of Blood Brain Barrier (BBB) – its loss is proposed to be a critical step in MCP-1-induced modulation of brain microvascular endothelial cells junctional protein expression and integrity [17023578]. Similarly, its downregulation in murine macrophages was shown to increase LPS-induced proinflammatory cytokine TNF-alpha and IL-6 production, but decrease anti-inflammatory cytokine IL-10 production [16357362].
Bordetella pertussis is a gram-negative bacteria, the causative agent of pertussis (whooping cough). Its toxin interferes with intracellular communication by affecting the functioning of regulatory G-proteins and preventing them from interacting with cell membrane receptors. The close involvement of G-protein activation in regulation of opening times of voltage gated calcium channels has been observed in many types of cells including neurons [8382734, 2560167], insulin-secreting pancreatic beta cells and catecholamine-releasing adrenal chromaffin cells [15488596, 1714959] (see GI, Neurotransmitters and Hormones).

In animal studies the changes that were observed with regards to permeability of BBB and cellular calcium overload were thought to play a central role in the pathogenesis of infectious brain edema induced by injection of pertussis bacilli in rat brain. Treatment with nimodipine was neuroprotective – it dramatically reduced the damage of brain edema by inhibiting the excess of calcium influx and reducing the permeability of BBB (see BBB). Pretreatment of neurons by MK-801, a NMDA channel antagonist was observed to inhibit delayed calcium influx [12659709, 9812737]. Similarly to Borna Disease Virus infection of neonatal mice (see above), the reaction to pertussis toxin/vaccine and occurrence of toxin-induced encephalitis appears to be mouse-strain dependant [6933900]. In addition, it was found that mice that overexpress monocyte chemotaxtractant protein-1 (MCP-1) at high levels show greater vulnerability to pertuss toxin and that the disruption of CC chemokine receptor 2 (CCR2) abolished both CNS inflammation and encephalopathy in this mouse model, identifying the central role of CCR2 in pertussis-induced encephalopathy [12486156] (see below on role of chemokine receptors in neurological disorders).

A significant association was shown between neurological disorders and pertussis toxin in humans [6786580, 9755273]. Residual levels of active pertussis toxin and endotoxin are likely to be a major contributors to the reactogenicity of whole cell pertussis vaccines, and new guidelines and limits have been established by WHO for active pertussis toxin in acellular pertussis vaccine [link]. In addition, it has also been proposed that permeability changes in the cerebral vessels may be involved in the evolution of the encephalopathy attributed to the use of the vaccine [12780, 12503649].

Various other types of bacteria and their toxins interfere with calcium homeostasis of the host cell. Bacterial lipopolysaccharide (LPS) activation of mouse microglia in vitro leads to elevated basal calcium along with attenuated calcium signaling in response to stimulation, translating into reduced ability of activated microglia to respond to an external stimulus. In addition, the rise in calcium levels underlie characteristic features of microglial activation, such as release of nitric oxide (NO) and several cytokines and chemokines. In the absence of available calcium the LPS-stimulated secretion of cytokines and NO is greatly reduced.

One of the proposed ways of bacteria-induced rises in basal calcium is through formation of toxin-created calcium channels on cell membrane required for bacterial cell invasion, although more recent line of evidence suggests direct modulation of existing LTCC channels and their function [3917612, 6510944, 16732377, 12736823]. Addition of calcium channel blockers, calmodulin antagonists and/or chelation of extracellular calcium significantly reduce bacterial entry into host cells in vitro [12761148, 8382566, 16151220]. This same effect was also noted in invasion of host cells by Toxoplasma gondii [9309395, 15591836], a parasitic agent capable of passing on to humans and causing low grade encephalitis followed by neurological and behavioural modifications [16000166].

Activation of various chemokine receptors expressed on cell surface by bacterial agents is proposed as another mechanism behind bacteria-induced rises in cell calcium levels (see below). Apart from the actions of their LPS, other bacterial membrane components are also known to act on various chemokine and toll-like receptors and influence their expression in different cell types, with CCR2, CCR5, TLR2 all being implicated [10559223, 14733721,
TLR2 for example is essential for the recognition of peptidoglycan and lipoprotein/lipopeptides that are present in cell walls of a variety of micro-organisms, and rodent studies show that mice lacking this receptor do not exhibit inflammatory response when exposed to these agents [12697090].

Encaphalitis caused by mycoplasma pneumoniae, a lung-infecting bacteria capable of causing neurological manifestation, is characterised by elevated levels of several cytokines and beta-chemokines in the lungs and the CNS [10441731, 16087054]. Changes in the intracellular calcium levels, most probably mediated through activation of toll-like receptors TLR2 and -6, are thought to underlie the systemic inflammation and pathologenesis of mycoplasma genus of bacteria [16154916, 15312143]. In addition to the involvement of G-protein coupled receptors and plasma calcium channels, subsequent release of calcium ions from endoplasmic reticulum has also been observed [11953388].

Lipoprotein found on Treponema pallidum, causative agent of syphilis and implicated as one of the possible causative or contributing agent in autism (see above), induces CCR5 on human monocytes and enhances their susceptibility to infection by HIV [10608777]. Absence of the chemokine receptor CXCR2 results in reduced inflammation induced by Borrelia burgdorferi, another spirochete bacteria and the causative agent in Lyme disease and neuroborreliosis [15760769]. Similarly, infection of nul CXCR2 mice resulted in a significant decrease in susceptibility to development of experimental Lyme arthritis, suggesting that chemokine-mediated recruitment of neutrophils into the infected joint is a key requirement for the development of this condition [12847259].

Prion encelopathies are characterised by neurotoxicity, neurodegeneration and microglial activation induced by mutated forms of the prion protein and the amyloid beta precursor protein (Abeta), and are thought to be mediated through their effects on calcium conductances through L-type calcium channels [9914452, 14622132, 10817932]. The so called channel hypothesis of Alzheimer’s disease proposes that Abeta proteins accumulate in the brain and damage neurons by forming ion channels [12128087]. This hypothesis is further supported by neuroprotective effects shown by some calcium channel blockers in experimental Abeta neurotoxicity [17051460]. In addition, the involvement of presenilin enzymes in Alzheimer’s disease is hypothesised to be unrelated to their role in formation of amyloid peptides, but more likely linked to ability of mutated forms of presenilins to directly disturb cellular calcium homeostasis, leading to excessive levels of intracellular calcium, formation of ROS and cellular death [9614221] (see Related_Conditions).

**Chemokine receptors and calcium homeostasis in the brain**

Chemokines belong to a family of chemotactic cytokines that direct the migration of immune cells towards sites of inflammation. They mediate their biological effects by binding to chemokine receptors on the cell surface. In addition to cytokines and chemokines, various other agents, like viral and bacterial proteins are capable of binding to these receptors and influencing cellular metabolism through this mechanism (see above). Since both chemokines and their receptors as well as various infectious agents have been implicated in the pathophysiology of a number of autoinflammatory diseases, including those involving inflammatory processes in the brain and CNS, chemokine receptor antagonists have often been suggested as potential treatment agents.

Chemokine receptors and their ligands are thought to have regulatory functions in the normal nervous system, where they participate in cell communication and may play a role in the control of cerebellar neuron survival and development. Since many of these receptors are widely expressed by various cells belonging to both the immune and the nervous systems, they have been suggested to serve as a ‘bridge’ between the two. This is further supported by the observed crosstalk between chemokine and neuropeptide receptors, such as opioid, vasoactive intestinal peptide, or adenosine receptors, whereas activation of one often has inhibitory effect on the other [16204635] (see also Related_Channels). In addition, activation
and/or inhibition of these G-protein coupled receptors has a direct effect on functioning of membrane calcium channels and cellular calcium homeostasis.

In the CNS, activation of neuronal chemokine receptors by their ligands induces calcium transients and activates calcium cAMP-dependent gene transcription factor CREB (see Brain re its role in neuronal gene expression). Of note is that these effects were in some cases observed to be pertussis toxin-insensitive, indicating possible involvement of pathways that are not mediated by G-proteins [11958818]. Increases in calcium levels have also been observed in cultured embryonic and adult neural progenitor cells and embryonic neurons, with neurospheres grown from young mice exhibiting significantly larger increases in calcium following chemokine treatment than did neurospheres grown from older mice. It was suggested that chemokines may play an important role in control of progenitor cell migration in embryonic and adult brain [15048927, 11567789, 15465598] (see Brain re expression and function of calcium channels during brain development). In addition, the calcium/CREB-mediated upregulation of chemokine receptors mRNA has been observed in T lymphocytes, whereby activation of for example CXCR4 by viral proteins leads to increased viral infectivity [11874984, 14563373] (see Immune/Inflammation re proposed involvement of cellular calcium loading in reduced T-cells reponsiveness in autism).

Of special significance to developmental disorders could be the possible role played by chemokine receptor CCR2, as its involvement in neuronal communication and in particular cholinergic and dopaminergic neurotransmission has been observed. Its preferential ligand, chemokine MCP-1/CCL2 was shown to induce calcium transients in primary cultured neurons from various rat brain regions including the cortex, hippocampus, hypothalamus, and mesencephalon [16196033]. The near-universal induction of MCP-1 and CCL2 after neurological insult suggests that this chemokine plays a central role in the physiology of neuroinflammation – in animal studies chronic overexpression of MCP-1 in the CNS causes delayed encephalopathy and impaired microglial function in mice, and possible dysfunction of BBB has also been implicated. This experimental pertussis toxin-induced reversible mouse encephalopathy was completely dependant on MCP-1 overexpression, and mice lacking the MCP-1 gene showed absolute resistance [15857890]. Of note is that levels of MCP-1 were found in a postmortem examination to be significantly raised in brains and CSF (12-fold rise) of subject with autism compared to controls (see Immunity/Inflammation).

Similarly, overactivation of other chemokine receptors, as well as toll-like receptors, have been implicated in CNS inflammatory diseases [17151286, 15557205]. The presence of fractaline receptor CX3CR1 signalling has on the other hand recently been proposed as having protective effects against microglial neurotoxicity [16732273]

Apart from neurons, chemokine receptors are also widely expressed on brain glial cells, where their function modulates intracellular calcium signalling and calcium-regulated gene expression [15850665, 16547971, 12112372]. The abovementioned interactions between chemokine and opioid receptors and downstream effects on calcium-mediated pathways are thought to underlie the observed effects of opioid treatment and antagonism on viral infection and outcomes [15893700]. Cultured astrocytes simultaneously treated with opiates and viral protein Tat exhibit exaggerated increases in chemokine release, including MCP-1, RANTES and IL-6, an effect that is mediated via the modulation of MAPK and CREB signaling pathways, and that can be prevented by applying a mu-opioid receptor antagonist [15630704, 15893700, 17162664] (see also Related_Receptors).

**Genetic polymorphisms as predictors of inflammatory outcomes and chemokine receptors as treatment targets**

Several chemokine receptor gene variants have been demonstrated to have detrimental effect on HIV infection and progression to AIDS, and various CCR5 genotypes in children determine both speed of disease progression as well as the degree of neurological impairment [14624371, 9696841].
Similar chemokine receptor-related genetic polymorphisms play a role in vulnerability of gastrointestinal tract to infectious agents, and chemokines and their receptors are thought to be viable therapeutic targets for the treatment of inflammatory bowel disease. A good example is that genetic deficiency in the chemokine receptor CCR1 was shown to protect against acute Clostridium difficile toxin A enteritis in mice - the toxin induced in all mice a significant increase in ileal fluid accumulation, epithelial damage, and neutrophil infiltration, but with all parameters being significantly lower in CCR1 and MIP-1alpha knockout mice [11875005].

Targeting CCR5 and its ligands has been suggested as a possible immune-modulating strategy since it appears to be involved in recruitment of immature MHC class II dendritic cells to the site of inflammation [15790880] (see also Immunity/Inflammation). In the case of HIV infection, various agents capable of modulating expression of multiple chemokine receptors are currently being developed as novel antiviral strategies [12936978, 12936978, 7000001].

The blockade of CCR2, CCR5 and CXCR3 by a novel chemokine antagonist TAK-779 prevents experimental colitis in murine studies by inhibiting the recruitment of inflammatory cells into the mucosa [16000328]. TAK-779 is currently in clinical trial for human use approval and is expected to be used as treatment agent in HIV infection and possibly Inflammatory Bowel Disease (see Gastrointestinal for inflammation and other gastrointestinal issues in autism).

**Other issues for consideration**

Observations that a virus can act both as an immunosupressor and as a stressor, capable of activating the hypothalamic-pituitary-adrenal axis and increasing brain concentrations of tryptophan and norepinephrine catabolite should be given some consideration in the context of brain development [2756050, 3509812] (see Hormones and Maternal_Serotonin).

A series of murine and studies pointing to **links between viral infections and absorption and tissue/organ distribution of environmental toxins** may be of great relevance. It has been observed for example that the intestinal absorption of cadmium increases during a common viral infection - in the infected animals the absorption of this metal was increased by 70% at low doses and was tripled at high doses compared to non-infected animals. The increased absorption also enhanced the accumulation of cadmium in all studied organs [9630849]. Redistribution of trace elements already present in the body has been noted, with brain levels of mercury increasing twofold during viral infection [16327074]. Mercury was shown to change virally-induced myocarditis in a direction compatible with the development of chronic disease and allow increased persistence of virus, indicating that heavy metals may interact and adversely affect viral replication and development of inflammatory disease. Of note is that in the inflammatory lesions of mice exposed to mercury, the myocardial contents of calcium, manganese, and iron were significantly increased compared to controls [11314973]. The observed changes in the patterns of accumulation, excretion and toxicity of the environmental pollutants during common virus infection has been proposed to be due to down-regulation of detoxifying processes in favour of acute-phase protein synthesis in the host in response to infection [11846173].

With regards to latent versus active viral infection, **various factors are known to be capable of reactivating a latent virus, a process which appears to be calcium-dependent and involves elevations of cellular calcium levels** [12692270]. An estimated three quarters of diagnosed autism cases seem to fall into category of regressive autism – a sudden loss of previously acquired language and sociobehavioural skills, usually occurring between 18-24 months of age, following a period of normal development. The exact reason for this has been a subject of controversy, with parents and carers reports of regression following series of vaccination contrasted by epidemiological studies reporting no causal link, leading to recent proposals to study incidence of autism in unvaccinated populations. Of note is that vaccinations have been proposed as capable of triggering reactivation of latent virus
infections, via vaccine-induced immunomodulation [11103441]. This mechanism of an induced immune challenge having a trigger-like effect on latent viruses has been suggested to underlie development of CNS autoimmune diseases [11517396]. Pregnancy has been suggested as another immune-related event capable of triggering reactivation of latent viruses. Reactivation of HHV-6 seems common during pregnancy, and transfer of this herpesvirus to the fetus is estimated to occur in approximately 1% of pregnancies [10558965].

On the other hand, latent a viral infection, notably by herpesviruses, has also been shown to alter neuronal gene expression, including genes involved in neurotransmission and cellular excitability, and latent viruses are therefore proposed to play a possible role in etiology of chronic disease [12915567].

Finally, following recent discoveries of human chromosomal integration of herpesviruses and their sites, the possible role in etiology of viral integration in chromosomal abnormalities and various human pathologies, including autism, should not be completely discounted [15255601, 10477678, 16597897, 16341055, 4285072] (see also Genetic_Factors).
Maternal factors - pregnancy, calcium homeostasis and implications for development

Maternal viral infection during pregnancy has been hypothesised to alter fetal brain development indirectly - maternal inflammatory response was shown in vitro to interfere in neuronal development and CNS organisation [2273013, 12666123, 17029701]. In addition to cytokines and other inflammatory mediators, maternal antibodies have been suggested to play a possible role in some forms of neurodevelopmental disorders, as the mice injected with serum containing maternal brain antibodies exhibited changes in fields of exploration and motor coordination [12666123].

Another study found that prenatal exposure to antibodies from mothers of children with autism (MCAD) also produced neurobehavioral alterations in mice - displayed anxiety-like behavior on and had a greater magnitude of startle following acoustic stimulation. On a social interaction paradigm, adult mice had alterations of sociability. Pilot studies of immune markers in MCAD IgG-exposed embryonic brains suggest evidence of cytokine and glial activation. These studies demonstrate that the transplacental passage of IgG from MCAD is capable of inducing long-term behavioral consequences [19362378].

The role of antibodies to several neuronal and muscle ion channels in the etiology of neuromuscular transmission disorders is well known. For example the ability of anti-tissue antibodies to cause contraction of smooth muscle cells is thought to be due to their interaction and modulation of with plasma membrane calcium channels, with the most likely involvement of LTCC [8288445].

Congenital Heart Block (CHB) is a congenital heart condition caused by maternal autoantibody-mediated disturbance of LTCC in the heart of the newborn - maternal antibodies recognize human LTCC alpha(1C)-protein and functionally inhibit the expressed current. It should be noted that mothers of affected children frequently suffer autoimmune diseases, and that the fetal heart appears to be uniquely vulnerable and that the mother's heart is almost never affected with complete heart block despite exposure to identical circulating levels of autoantibodies [11427485].

As voltage-gated ion channels are expressed in the brain as well as at the neuromuscular junction and in the heart, it is often suggested that antibodies to some CNS channels could be associated with CNS disease. It can also be argued that a mechanism of similar nature to CHB could be at play in autism, where maternal autoantibodies could be affecting calcium channels and thus dysregulating ionic currents in neurons, glial or endothelial cells that line brain vessels.

Recent evidence has shown that antibodies directed against angiotensin II type I receptors are also highly associated with preeclampsia. It has been suggested that maternal antibodies could activate these receptors and subsequently plasma calcium channels, leading to an increase in intracellular calcium levels and to downstream activation of calcium signaling pathways [15381659, 12593997].

Plasma serotonin levels in mothers of ASD subjects was observed in one study to be significantly lower than in control mothers. Low maternal serotonin levels were proposed to be a risk factor for autism through effects on fetal brain development, especially in the light of a recent murine study showing that maternally-derived serotonin, as opposed to fetus-derived, is crucial for murine embryonic development [6939857, 17182745]. Worth noting is that lowered serotonin levels are a frequent feature of viral infections, since maternal viral infection and/or reactivation is a proposed risk factor in etiology of autism [16842443, 2771632, 9026369].

The mechanism behind the effects of serotonin on brain development and function is hypothesised to be at least in part linked to the inhibitory effects of serotonin on voltage-gated
calcium currents, most likely through activation of its 5-HT1A and possibly 5-HT2A receptors [11494406, 8118677, 11976386]. The importance of serotonin in promoting directed neuronal migration is hypothesised to be due to its G-protein-dependent modulation of voltage-gated calcium channels in the migrating cell [12401168]. Of interest here is that 5-HT1A agonists, in addition to reducing ischemic brain injury in vitro, have been proposed as possible agents for prevention of (virally-induced) neuro-psychiatric disorders (see AIDS ref above, also Infectious_Agents).

In the context of prenatal/maternal infection as a possible risk factor, worth noting is the greater occurrence of placental trophoblast inclusions observed recently in ASD individuals compared to controls [16806106], since infectious agents have been observed to persist and replicate in placental trophoblast cells, often causing their inclusions [2446593, 11743054, 6283965, 3918685]. In addition, human placental trophoblasts express voltage-gated calcium channels, and their activation induces secretion of corticotropin releasing hormone (CRH), thought to be closely linked to acute or chronic metabolic, psychological, physical or infectious stress during pregnancy. CRH hypersecretion has been associated to depression and anxiety-related disorders, anorexia nervosa and neurodegenerative diseases [10999834, 17127316]. For example it has been shown that exposure of animals to stressful experience (restrain stress) during pregnancy can exert effects on calcium ion channels of offspring hippocampal neurons and that such calcium channel disturbances may play a role in prenatal stress-induced neuronal loss and oxidative damage in offspring brain [17123551].
Thyroid function as a risk factor and involvement of calcium signalling

Familial autoimmune thyroid disease has recently been identified as a risk factor for regression in children with ASD and hypothyroid hormone deficiency in early development has been proposed to cause central nervous system damage that could lead to emergence of autistic symptoms [16598435, 1577897].

Thyroid hormone is essential for the development and maturation of the CNS in mammals. Deficiency of the hormone during development negatively affects performance in tasks of learning and memory in rodents, and was shown to cause abnormalities of the CNS such as incomplete maturation of neurons and glial cells, reduction in synaptic densities and myelination deficits. Rodent with mild and transient neonatal hypothyroidism has been proposed as a novel model for autism.

It has been speculated that behavioral deficits associated with developmental hypothyroidism are due to enhanced calcium influx through LTCC [15673845] and/or impairments of the calcium ATPase activity during critical stages of brain development [16293371]. Amongst other things thyroid hormone regulates neurotransmitter release in neonatal rat hippocampus and hypothyroid rats exhibit an increase in the calcium-dependent neurotransmitter secretion (see Neurotransmitters), an effect that could be reversed by administering thyroid hormone [11882369]. In additon, a recent study demonstrated that thyroid hormone insufficiency during early postnatal period permanently alters expression of calcium binding protein parvalbumin (PV) in GABAergic interneurons and compromises their inhibitory function [17008398].

In cardiac tissue, hypothyroidism impairs the early postnatal maturation of voltage gated calcium channels as regards both their distribution and mRNA expression. L-type and T-type calcium channels, as well as the ryanodine channel are amongst those affected by actions of thyroid hormone. Hyperthyroidism is associated with increases in cardiac LTCC currents, and changes in thyroid status markedly influence cardiac contractile and electrical activity. These mechanism are thought to be the likely causes of development of heart failure in hyperthyroidism [9539869, 16238192, 12914768, 10710339].
Conclusion

It is proposed that dysregulated calcium signalling, mainly through disturbances in VGCC function and downstream signalling pathways, is the central molecular event that leads to pathologies of autism.

Various genetic and external factors are capable of perturbing calcium homeostasis during critical stages of development. As concordance of autism in monozygotic twins is less than 100% and the phenotypic expression of the disorder varies widely, it is concluded that non-genetic mechanisms could greatly contribute to the onset of autism.

Environmental influences and risk factors mentioned in this paper, such as perinatal hypoxia and exposure to infectious agents and various environmental toxins, are proposed as possible etiological agents. Furthermore, genetic polymorphism related to immune function and inflammatory reactions, such as MIF, and expression of chemokine receptors, such as CCR2 and others, would make for additional risk factors, raising vulnerability following exposure in critical stages of development. A combination of such influences, or combinations with inherited mutations in calcium signalling pathways or closely linked proteins, would account for the complexity of autistic phenotype and severity of symptoms.

Involvement of chemokines, chemokine receptors and their signalling in inflammatory and neurological disease has become more evident in recent years. It is becoming increasingly clear that, beyond their involvement in neuroinflammation, these proteins play an important role in brain development and function, due in great part to their ability to modulate functioning of ion channels. In addition to neurons and brain glia, chemokine receptors are widely expressed on immune and endothelial cells, and have been proposed to serve as a bridge linking the immune and nervous systems. Targeting specific chemokines and chemokine receptors, either directly or indirectly through activation of related opioid, adenosine, and vasointestinal peptide receptors, is a novel therapeutic strategy in both inflammatory and infectious disease. Apart from AIDS-dementia, Alzheimer's disease and the neuroinflammation associated with multiple sclerosis, desensitisation and/or antagonism of specific chemokine receptors have been proposed as treatment targets in inflammatory disorders of gastrointestinal tract. Polymorphisms in genes that encode these receptors are now known to determine susceptibility to neurological dysfunction in HIV-infected individuals, and special attention should be paid to these factors considering that the symptoms and onset of AIDS-dementia in children are often identical to more common forms of autism [16101548, 16204635, 11456467, 16594639].

Lastly, antibodies to voltage-gated calcium channels, as well as to proteins that are able to modulate their function, such as for example maternal antibodies directed against angiotensin II type I receptors, could also play an etiological role in autism. Possibility of perinatal infection that develops into latent, subclinical infection, and potential reactivation following an external stressor, such as vaccination, should not be discounted. Apart from the risk of reactivation, persistent low-level infection could have major implications during development due to possible modulation of calcium signalling pathways in host cell.
Appendix i: Retroviruses and Autism

note: in a recent small scale preliminary study by Whittemore Peterson Institute around 40% of children with autism tested positive for XMRV murine retrovirus DNA, with nearly 60% showing presence of antibodies to the virus. (the presence of XMRV retrovirus in general healthy population is around 4%). Larger scale study is currently under way.

**HIV infection in children - neurodevelopmental (autistic) outcomes and clinical pathologies - and their correlations to idiopathic autism**

There is a striking correlation between neurodevelopmental symptoms often found in children infected with HIV virus and those children diagnosed with idiopathic Autism Spectrum Disorders (of unknown aetiology). Furthermore, the underlying clinical pathologies found in HIV-positive children are in many ways identical to biomedical pathologies found in children diagnosed with ‘common’ idiopathic autism.

The mechanisms of HIV-injury on host cellular systems have been identified in recent years and these pathologies very often closely match those found in autism, such as microglial activation, cellular calcium overload, mitochondrial dysfunction, oxidative stress, vasoconstriction, glutathione depletion, chronic inflammation of gastrointestinal and central and peripheral nervous systems etc (see list below).

Many treatment agents used in treating autism, weather with studied and proven beneficial effects or anecdotal reports of reducing autistic symptoms in some affected individuals, have antiretroviral mode of action and have been shown to inhibit the viral activity and/or reduce HIV viral load.

**Neurodevelopmental findings in HIV infected children**

Impairments in language, especially expressive language, behavioural symptoms: irritability, lack of social skills, repetitive actions (rocking etc).

Severity of autistic symptoms in HIV positive children is correlated to levels of the viral load/replcation, as well as CD4+ levels. Autistic symptoms – deficits in language, behaviour and social skills – in HIV infected children often recover upon administration of single or combination antiretroviral treatments, at least to some degree. Sometimes recovery is complete, with total remission of autistic symptoms.

HIV infected children sometimes develop normally and regress later, usually between 1.5-2 years of age. This is linked to increased HIV viral load.

Latent retrovirus/HIV can be reactivated by vaccinations. In addition to this, live virus vaccines, especially MMR, often come with a warning for HIV infected individuals with low CD4+ counts – inability to mount appropriate immune responses results in vaccine virus persistence. For example polio vaccine strain has been found in gastrointestinal tract of vaccinated individuals. No antibody production to Dtp or measles live virus vaccine. These findings have lead to proposals that both immunotherapy and vaccination of HIV-infected individuals should be accompanied by administration of an antiviral drug(s). In addition, it is suspected that exposure to antigenic stimulation through vaccinations may enhance the susceptibility of uninfected subjects to HIV-1.
(reactivation by endogenous retroviruses by external stressors, including vaccinations, has been proposed as causal in other autoimmune diseases, such as multiple sclerosis and arthritis)

**Gastrointestinal findings in HIV positive children parallel gastrointestinal abnormalities found in idiopathic autism**

Leaky gut and malabsorption of nutrients

Dysregulated production of digestive enzymes (impaired pancreatic function)

Abnormal immune reactions to gliadin and casein

Lactose intolerance

Sugar intolerance

Inability to digest complex carbohydrates

Inability to absorb fats and proteins

Gastrointestinal pathogen overload: secondary intestinal viruses, bacterial overload.

Abnormal immune reactivity to candida albicans.

**Others**

Impaired fine and gross motor skills in HIV positive children

Impaired sensory – auditory and visual processing

Subclinical hypothyroidism (in adults, no data on children)

**Pathological mechanisms in HIV infection**

HIV retrovirus causes calcium overload and mitochondrial dysfunction (also found in idiopathic autism)

HIV causes oxidative stress and glutathione depletion (also found in autism)

HIV causes microglial activation and inflammation (also found in autism)

HIV combined with bacterial agents causes breakdown of the blood brain barrier (bbb breakdown suspected in autism)

HIV causes glutamate exitotoxicity (dyregulated GABA/glutamate mechanisms observed in autism)

HIV causes vasoconstriction - tightening of blood vessels that supply oxygen to brain (observed in autism)

HIV inhibits methylation (abnormal methylation found in autism)
Many modalities currently used for treating autism have proven or suspected antiretroviral effects

- Chelation of metals inhibits HIV virus integration into human DNA. Retroviruses in general are desintegrated by chelation agents in vitro. Several chelators have been patented as antiretroviral agents. Several agents with chelating properties, such as alpha lipoic acid (ALA) and NAC have been shown to reduce viral load in HIV positive individuals.
- Tetracycline antibiotics (one currently on trial for autism) inhibit HIV in vitro through same mechanism as chelation agents.
- HIV is inhibited by glutathione and agents that raise glutathione
- Acyclovir/valacyclovir (antiviral agent with anti-herpevirus activity, with anecdotal reports of amelioration of autistic symptoms) has been shown to reduce HIV viral load in HIV positive individuals. The mechanisms are not clear.
- Hyperbaric oxygen has been shown to inhibit HIV and reduce viral load.
- Pancreative enzymes trial showed beneficial effect in HIV positive.
- Methylation agents such as cobalamins and SAMe directly inhibit HIV activity and maintain its latency.

References


----------


Human immunodeficiency virus-type 1 replication can be increased in peripheral blood of seropositive patients after influenza vaccination. O'Brien WA et al Blood. 1995 Aug 1;86(3):1082-9.

Measles/MMR vaccine for infants born to HIV-positive mothers [Intervention Protocol], B Unnikrishnan et al, The Cochrane Library 2009, Issue 1


Human exogenous and endogenous retroviruses in autoimmunity. Christensen, T., book chapter - Recent research developments in immunology. Vol. 7 Editors: Pandalai, S. G.

Endogenous Retroviruses and Human Neuropsychiatric Disorders – Book Retrotransposition, Diversity and the Brain Robert H. Yolken et al


See Infectious Agents for further references to mechanisms behind HIV-induced neurological dysfunction such as cellular and mitochondrial calcium overload in the brain (also present in 'common' autism)


Advances in two-metal chelation inhibitors of HIV integrase Authors: Johns, Brian A; Svolto, Angilique C Source: Expert Opinion on Therapeutic Patents, Volume 18, Number 11, November 2008 , pp. 1225-1237(13)


Appendix ii:

Summary of abnormal biomedical findings in autism

Below is a partial summary of some of the recent research into abnormal biological markers in people with autism. These findings are organized under nine headings: Neurological abnormalities; Gastrointestinal problems; Neurotransmitter abnormalities; Hormonal abnormalities; Auditory, visual, tactile and oral sensory processing disorders and motor difficulties; Reduced cerebral blood flow and cerebral edema; Abnormality of the immune function and chronic inflammation; Oxidative stress; and Mitochondrial dysfunction.

Neurological abnormalities in autism

Various neuropathological and MRI studies have pointed to the following neurological abnormalities in autism:

- significantly elevated calcium levels in autistic brains compared to controls, followed by elevations of mitochondrial aspartate/glutamate carrier rates and mitochondrial metabolism rates (18607376) also see Mitochondrial dysfunction below
- abnormal neuronal migration in both brainstem and cerebellum and disorganised columns of the cerebral cortex.
- significant reduction in granular and Purkinje cell numbers, often accompanied by gliosis (proliferation of astrocytes in damaged areas of the brain).
- marked active/ongoing neuroglial activation and neuroinflammation (see Immunity and Inflammation below).
- abnormalities of cortical development has been observed in some cases, including areas of increased cortical thickness, high neuronal density, neuronal disorganization and poor differentiation of neurons.
- abnormalities in brain size and volume, recently linked to increased tissue water content in brain matter
- reduced blood flow to parts of the brain
- abnormalities in the size and density of neurons and less dendritic branching, with increased neuron density and shorter connecting fibers, pointing to delays in neuronal maturation.
- autoantibodies to brain proteins, notably to myelin basic protein, neuron-axon filament protein and glial fibrillary acidic protein (see Immunity/Inflammation).

(20198484, 15546155, 18435417, 15749244, 9619192, 18607376, 9758336, 16819561, 14519452, 16214373, 6924017)

Gastrointestinal abnormalities in autism

Individuals with autistic spectrum disorders tend to suffer from various, sometimes severe gastrointestinal problems. Children with ASD are suspected to have a higher rate of gastrointestinal (GI) symptoms when compared with children of either typical development or another developmental disorder, although large-scale studies are yet to be carried out and the significance of the association between many gastrointestinal pathologies and autism is yet to be confirmed.

A recent consensus report on evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with autism concluded that care providers should be aware that problem behavior in patients with ASDs may be the primary or sole symptom of the underlying medical condition, including gastrointestinal disorders, and guidelines have been issued on evaluation and treatment of common gastrointestinal problems in children with ASDs, such as abdominal pain, chronic constipation, and gastroesophageal reflux disease (20048083, 20048084).
The most frequent complaints are chronic constipation and/or diarrhea (frequently accompanied by indigested or partially digested food in stools), gaseousness, and abdominal discomfort and distension (14523189). Decreased sulfation capacity of the liver, pathologic intestinal permeability, increased secretory response to intravenous secretin injection, and decreased digestive enzyme activities have been reported in many children with autism. Treatment of digestive problems is reported to have positive effects on autistic behaviour in some individuals (8888921, 12010627, 1176974).

Defects of innate immune responses in ASD children with GI problems have been detected, and intestinal pathology, including ileocolonic lymphoid nodular hyperplasia (LNH) and mucosal inflammation, with enhanced pro-inflammatory cytokine production, have been noted in various studies. A majority of the children were shown to have chronic swelling of the lymphoid tissue lining the intestines, particularly near where the small and large intestines meet, and chronic inflammation of the large intestine. Secondary eosinophilic colitis has also been observed in autism. There is a consistent profile of CD3+ lymphocyte cytokines in the small and large intestinal mucosa of these ASD children, involving increased pro-inflammatory and decreased regulatory activities (16494951, 15741748, 11007230, 15622451, 20068312). The mucosal immunopathology in children with autism is reported to be suggestive of autoimmune lesion and is apparently distinct from other inflammatory bowel diseases (11986981, 15031638)). See also Immune and Inflammation.

One study examining histologic findings in children with autism revealed high incidence of grade I or II reflux esophagitis, chronic gastritis and chronic duodenitis. The number of Paneth cells in the duodenal crypts was significantly elevated in autistic children compared to controls. Low intestinal carbohydrate digestive enzyme activity was reported in over half of the children with autism, although there was no abnormality found in pancreatic function. Seventy-five percent of the autistic children had an increased pancreatic-biliary fluid output after intravenous secretin administration, suggesting an upregulation of secretin receptors in the pancreas and liver (10547242).

There are also reports of prominent epithelium damage (11241044), and of significant alterations in the upper and lower intestinal flora of children with autism. One striking finding was complete absence of non-spore-forming anaerobes and microaerophilic bacteria from control children and significant numbers of such bacteria from children with autism. The faecal flora of ASD patients was found to contain a higher incidence of the Clostridium histolyticum group of bacteria than that of healthy children (1552850, 12173102, 16157555).

On the other hand a low-grade edotoxemia has recently been observed in autism. Compared with healthy subjects, serum levels of bacterial endotoxin were significantly higher in autistic patients and inversely and independently correlated with the severity of autism symptoms, noting the need for further studies to establish whether increased endotoxin may contribute to the pathophysiology of inflammation and behavioural and social impairments in autism (20097267). Presence in blood of endotoxins produced by gastrointestinal pathogens is indicative of impaired gastrointestinal permeability and present in chronic infectious and inflammatory conditions – a good example is the association of HIV-infection with increased gut permeability and microbial translocation, evidenced by increased circulating lipopolysaccharide (LPS) levels, mirroring the above-mentioned findings in autism (17720995).

**Neurotransmitter abnormalities in autism**

Abnormalities in neurotransmitter systems have frequently been recorded in autism. Clinical observations include both elevated and lowered levels of various neurotransmitters compared to controls, including alterations in monoamine metabolism [3654486, 2653386, 3215884], neurotransmitter peptides [9018016, 9315980], with considerably raised levels of beta-endorphin (for vasopressin/oxytocin see Hormones) and altered activities of cholinergic receptors, with binding of muscarinic M(1) receptor being up to 30% and that of nicotinic receptors being 65%-73% lower in the autistic group compared to controls [11431227].
Postmortem brain examination noted abnormalities of the glutamate neurotransmitter system in autism, with specific abnormalities in the AMPA-type glutamate receptors and glutamate transporters in the cerebellum [11706102]. Expression of several types of GABA receptors is altered in brains of subjects with autism, with levels being significantly reduced in autism compared to controls [18821008, 19002745].

Dysregulations of serotonergic systems in particular have been documented, such as abnormalities in brain serotonin synthesis, with significant reductions in synthesis capacity compared to controls [10072042, 9382481], while at the same time plasma levels of serotonin and free tryptophan appear to be on average 30-50% percent higher in individuals with autism [6204248]. Autoantibodies to serotonin receptors [9067002] and reduced receptor binding have also been recorded [16648340]. Of note is that one study found correlation of elevated plasma serotonin levels and the the major histocompatibility complex (MHC) types associated with autism [8904735].

Relative to expression and function of nicotinic receptors in autism, significantly lowered binding of some agonists to nicotinic receptors has been observed. For example binding of the agonist epibatidine in cortical areas to was up to 73% lower in autism group compared to controls. As with serotonin receptors, some of the nicotinic receptors have been noted to be significantly permeable to calcium and so able to regulate several neuronal processes. Of interest are the preliminary reports of therapeutic action of galantamine in autism [15152789, 17069550], considering that neuroprotective actions of galantamine are thought to be linked to its modulation of nicotinic receptors [12649296].

A postmortem study revealed greatly reduced levels of glutamic acid decarboxylase (GAD) 65 and 67 kDa proteins in several areas of the brains of individuals with autism [12372652]. This was confirmed by more recent results that showing GAD67 mRNA level reduced by 40% in the autistic group when compared to controls [17235515]. Another study found serum levels of glutamate in the patients with autism were significantly higher than those of normal controls [16863675].

**Hormonal abnormalities in autism**

Abnormalities in hormonal metabolism are frequently observed in individuals with autism, with several studies observing abnormal levels of many hormones and their receptors compared to healthy controls, as well as abnormal hormonal secretion rhythms [2713159, 1904373, 12959423, 10808042].

For example the analysis of the Hypothalamic-Pituitary-Adrenocortical (HPA) system responses observed more variable circadian rhythm as well as significant elevations in cortisol following exposure to a novel stimulus in children with autism compared to controls. This exaggerated cortisol response is indicative of dysfunction of the HPA system in autism (16005570). Over-reaction of the endocrine system to insulin stress in autism has been recorded in another study, whereas the experimental stress of insulin-induced hypoglycemia showed slower recovery of blood glucose, much faster cortisol response and elevation of growth hormone levels compared to controls (1176974, 2870051). Low levels of insulin-like growth factor-I (IGF-I) in cerebrospinal fluid have also been observed (16904022).

Metabolic disorders of serotonin and dopamine systems have been suggested in autism, with approximately thirty percent of individuals with autism exhibiting high levels of serotonin, simultaneous with lowered levels of melatonin (see Neurotransmitters). Melatonin is converted from serotonin by several enzymes of the pinealocytes in the pineal gland, including 5-HT N-acetyl transferase and 5-hydroxyindole-O-methyltransferase. Results of the studies looking at sleep disturbances in autism suggest that both dyssomnias and parasomnias are very prevalent in the disorders - people with autism frequently experience sleep disorders and exhibit atypical sleep architecture (10722958, 15705609, 17001527). Further evidence of dysfunction of pineal endocrine system in autism was obtained by looking at alterations of the light and dark circadian rhythm of melatonin, where none of autistic patient showed a normal
melatonin circadian rhythm, together with once again significantly lower levels of this hormone (11455326).

Leptin is a hormone linked to melatonin that plays an important role in amongst other things regulation of appetite and metabolism. Results from a recent study have demonstrated significant differences in leptin concentrations between children with autism and controls (17347881).

**Auditory, visual, tactile and oral sensory processing disorders and motor difficulties in autism**

Individuals with autism often present with auditory, visual, tactile and oral sensory processing disorders, as well as various forms of motor difficulties, including dyspraxia (occasionally linked to low muscle tone), dystonia (involuntary, sustained muscle contractions) and ataxia (16940314, 17016677, 15514415, 16903124, 12639336). Visual disturbances in autism often include abnormalities of colour perception (16598434) and weak visual coherence. Retinal dysfunction in autism has been suggested, as well as deficits in visual processing in dorsal cortex (3341467, 15958508). Abnormal pain perception is sometimes present in autism, as well as self-injurious behaviour. Dyspraxia is a disorder of coordination that can also be described as a difficulty with planning a sequence of coordinated movements, or in the case of ideo-motor dyspraxia, a difficulty with executing a known plan. Various areas of difficulty can include speech and language, fine motor control (eg handwriting or holding pencil in a correct way), poor spatial awareness and timing and balance of body movements and difficulty combining movements, poor physical play skills (throwing and catching a ball) and difficulty in manipulating small objects. Ataxia refers more specifically to a failure of muscle control in limbs, often resulting in a lack of balance and coordination and abnormal gait.

**Reduced cerebral blood flow and cerebral edema in autism**

Results of several studies have shown abnormal platelet reactivity and altered blood flow in children with autism. Following these findings it has been suggested that platelet and vascular endothelium activation could be one of the contributing factors to the development and clinical manifestations of the disorder (16908745). Relative to this the following case reports are of particular interest, both describing cases of inflammation of brain blood vessels resulting in loss of language and emergence of symptoms of autism. In both cases administration of nicardipine lead to recovery of language and behaviour (1373338, 11008286).

PET and SPECT scans in autistic children show a decreased cerebral blood flow in some regions of the brain (12077922, 10960047, 7790938) and cerebral water content was found to be raised in brain grey matter in children with autism (16924017). A model has been suggested in which the observed gray matter abnormality could be inflammatory (see Immunity-Inflammation section below). This finding of cerebral edema at the same time offered an alternative explanation for enlarged brain size in autism, which up to then had been hypothesised to be due to lack ‘pruning’ of neurons during development.

**Abnormality of the immune function and chronic inflammation in autism**

Results of numerous studies point to an abnormality of the immune function in autism, as well as active, ongoing inflammation in the GI tract, the brain and the cerebrospinal fluid (CSF).

A recent study by Vargas et al (15546155) investigated the presence of immune activation in postmortem brain specimens and CFS from subjects with autism. The authors found active neuroinflammation in multiple areas of the brain, for example in the cerebral cortex and white
Autistic children were found to have significantly higher antibody seropositivity compared to controls, and also had significant positive associations with disease severity, mental retardation, and electroencephalogram abnormalities. Another investigation into inflammatory markers in the brain tissue of patients with autism revealed significantly increased levels of several proinflammatory cytokines (TNF-alpha, IL-6 and GM-CSF, IFN-gamma, IL-8). The Th1/Th2 ratio was also significantly increased in ASD patients, suggesting that localized brain inflammation and autoimmune disorder may be involved in the pathogenesis of ASD (19157572).

Various serological findings further confirm the presence of immune system dysregulation and active inflammation in autism - raised levels of proinflammatory cytokines have often been observed in blood of patients with autism, with significant increases of IFN-gamma, IL-6 and TNF-alpha. These results are followed by findings of decreased peripheral lymphocyte numbers, incomplete or partial T cell activation following stimulation, decreased NK cells activity, dysregulated apoptosis mechanisms, imbalances of serum immunoglobulin levels, increased numbers of monocytes and abnormal T helper cell (Th1/Th2) ratio, with a Th2 predominance, and without the compensatory increase in the regulatory cytokine IL-10 (16698940, 16360218). It is of interest to note that, following increased levels of TGF-beta1 in brain specimen as observed by Vargas et al, this cytokine was found to be significantly lower in the blood of adult patients with autism compared to controls (17030376).

Another relevant observation is the elevation of cerebrospinal fluid levels of TNF-alpha compared to its serum levels in subjects with autism. The observed ratio of 53.7:1 is significantly higher than the elevations reported for other pathological states for which cerebrospinal fluid and serum tumor necrosis factor-alpha levels have been simultaneously measured (17560496).

It has been suggested that prenatal viral infection might dysregulate fetal immune system, resulting in viral tolerance in autism (139400). Studies showing altered T-cell subsets raised suspicions about possible autoimmune aspects of autism and several studies pointed to association of the risk of autism to immune genes located in the human leukocyte antigen (HLA) (16720216, 15694999). The immune system uses the HLAs to differentiate self cells and non-self cells and some of them are linked with higher risk of autoimmune disorders. Animal studies show that behavioural changes follow onset of autoimmune disease and can be reversed through immune-suppressive treatments (8559794), and although various autoantibodies to brain antigens have been observed in autism, the results of those studies are often conflicting. In addition to the inconsistent findings the question has been raised as to whether those autoantibodies would be pathogenic contributors or mere consequences of the the disorder. Of note in this context is the finding pointing to possible association of virus serology and brain autoantibodies in autism (9756729). One study found that children with autism had a significantly higher percent seropositivity of anti-nuclear antibodies. Anti-nuclear antibody seropositivity was significantly higher in autistic children with a family history of autoimmunity than those without such history, and also had significant positive associations with disease severity, mental retardation, and electroencephalogram abnormalities (19135624). Autistic children were found to have significantly higher serum anti-myelin-
associated glycoprotein antibodies than healthy children. Family history of autoimmunity in autistic children was significantly higher than controls. Anti-myelin-associated glycoprotein serum levels were significantly higher in autistic children with than those without such history (19073846).

A study looking at several markers of concomitant autoimmunity and immune tolerance found highly elevated circulating IgA and IgG autoantibodies to casein and gluten dietary proteins in autism sample compared to controls. Circulating anti-masles, anti-mumps and anti-rubella IgG were positive in only 50%, 73.3% and 53.3% of autistic children previously immunised by MMR, as compared to 100% positivity in the control group. Anti-cytomegalovirus CMV IgG was also investigated and was positive in 43.3% of the autistic children as compared to 7% in the control group (17974154).

A recent study found polyomavirus infection in postmortem autistic brains BKV, JCV, and SV40 viruses were significantly more frequent among autistic patients compared to controls [20345322].

Also worth noting is the finding that a large subset of subjects with autism shows evidence of bacterial and/or viral infections not present in age-matched controls [17265454], as well as a reduced response to vaccine antigens, especially Bortedella pertussis [link]. In addition, there appears to be a correlation between virus serology and brain autoantibodies in autism.

**Oxidative stress in autism**

Oxidative stress is defined as an imbalance between pro-oxidants and anti-oxidants, resulting in damage to cell by reactive oxygen species (ROS). During times of environmental stress ROS levels can increase dramatically which can result in significant damage to cell structures, especially in absence of anti-oxidant defences, such as the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase or antioxidant vitamins A, C and E and polyphenol antioxidants. There is mounting evidence that abnormalities of ROS and nitric oxide (NO) may underlie a wide range of neuropsychiatric disorders. Abnormal methionine metabolism, high levels of homocysteine and oxidative stress are also generally associated with neuropsychiatric disorders. NO signalling has been implicated in a number of physiological functions such as noradrenaline and dopamine releases. It is thought to have neuroprotective effects at low to moderate concentrations, but excessive NO production can cause oxidative stress to neurons thus impairing their function.

Studies comparing the level of homocysteine and other biomarkers in children with autism to controls showed that in children with autism there were higher levels of homocysteine, which was negatively correlated with glutathione peroxidase activity, low human paraoxonase 1 arylesterase activity, suboptimal levels of vitamin B 12 (16297937, 12445495) and increased levels of NO (12691871, 14960298).

Lipid peroxidation was found to be elevated in autism indicating increased oxidative stress. Moderate to dramatic increases in isoprostane levels (16081262, 16908745), decreased levels of phosphatidylethanolamine and increased levels phosphatidylserine (16766163) were observed in children with autism as compared to controls. Levels of major antioxidant proteins transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein) were found to be significantly reduced in sera of autistic children. A strong correlation was observed between reduced levels of these proteins and loss of previously acquired language skills (15363659).

Another study measured levels of metabolites in methionine pathways in autistic children and found that plasma methionine and the ratio of S-adenosylmethionine (SAM) to S-adenosyl-homocysteine (SAH), an indicator of methylation capacity, were significantly decreased in the autistic children relative to controls. In addition, plasma levels of cysteine, glutathione, and the ratio of reduced to oxidized glutathione, indicative of antioxidant capacity and redox homeostasis, were significantly decreased in autistic group. The same study evaluated common polymorphic variants known to modulate these metabolic pathways in 360 autistic
children and 205 controls. Differences in allele frequency and/or significant gene-gene interactions were found for relevant genes encoding the reduced folate carrier (RFC 80G), transcobalamin II (TCN2 776G), catechol-O-methyltransferase (COMT 472G), methylenetetrahydrofolate reductase (MTHFR 677C and 1298A), and glutathione-S-transferase (GST M1) (16917939).

Oxidative damage in autism is also associated with altered expression of brain neurotrophins critical for normal brain growth and differentiation. An increase in 3-nitrotyrosine (3-NT), a marker of oxidative stress damage to proteins in autistic cerebella has been reported. Altered levels of brain NT-3 are likely to contribute to autistic pathology not only by affecting brain axonal targeting and synapse formation but also by further exacerbating oxidative stress and possibly contributing to Purkinje cell abnormalities (19357934).

A study looking into cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism found that, compared to controls, autism LCLs exhibit a reduced glutathione reserve capacity in both cytosol and mitochondria that may compromise antioxidant defense and detoxification capacity under prooxidant conditions (19307255).

**Mitochondrial dysfunction in autism**

Mitochondrial dysfunction with defects in oxidative phosphorylation has been suspected in autism and several recent findings that show abnormalities in mitochondrial enzyme activities that support hypothesis. Postmortem examination of autistic brains revealed significantly elevated calcium levels in autistic brains compared to controls, followed by elevations of mitochondrial aspartate/glutamate carrier rates and mitochondrial metabolism and oxydation rates (18607376, also see Brain and Oxidative Stress sections above).

When compared to controls autistic patients show significantly lower carnitine levels, followed by elevated levels of lactate, aspartate aminotransferase, creatine kinase and significantly elevated levels of alanine and ammonia (16566887, 15739723, 15679182). A pilot study investigating brain high energy phosphate and membrane phospholipid metabolism in individuals with autism found decreased levels of phosphocreatine and esterified ends (alpha ATP + alpha ADP + dinucleotides + diphosphosugars) compared to the controls. When the metabolite levels were compared with neuropsychologic and language test scores, a common pattern of correlations was observed across measures in the autistic group, wherein as test performance declined, levels of high energy phosphate compounds and of membrane building blocks decreased, and levels of membrane breakdown products increased. The authors concluded that the results of the study provided tentative evidence of alterations in brain energy and phospholipid metabolism in autism that correlate with the level of neuropsychologic and language deficits (8373914). This was further confirmed by another study finding the impairment of energy metabolism in autistic patients which could be correlated to the oxidative stress (19376103).

Also see above - Oxidative Stress