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

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Introduction

Calcium is one of the most important second messenger molecules used by living cells. Signalling carried out by calcium ions plays an important role in many cellular processes and because of its universal nature, disruption of calcium homeostasis under pathological conditions can have numerous consequences.

Due to its importance, calcium homeostasis is a tightly regulated event and many molecules and subcellular structures are involved in maintaining its optimal levels and pathways. Calcium enters the cell from extracellular space through plasma membrane, and once within the cell its levels are regulated by various intercellular stores, pumps, and buffer proteins. One way of entry into the cell is through voltage-gated calcium channels (VGCC) on cellular plasma membrane. Calcium entry through VGCC is an important physiological event, and proper functioning of these channels is crucial in many cellular processes. VGCC are expressed in many diverse cells of the human body and their pharmacological properties are independent of the cell type where they reside.

L-type calcium channels (LTCC) are voltage-gated calcium channels that are ubiquitously expressed in the cells of the central nervous system (CNS), the immune system and the gastrointestinal tract, amongst others. During development LTCC are highly expressed in the brain, and their optimal functioning is of central importance in many aspects of embryonic and postnatal brain development, including neuronal gene expression and differentiation, growth, branching, migration, and structural organisation of developing neurons. Calcium influx through these channels is directly involved in secretion of neurotransmitters and hormones, and plays a pivotal role in development of motor coordination and sensory processing. Furthermore, calcium signalling is the molecular mechanism of integration of neural circuits in the CNS, and is able to directly moderate electrical activity and excitability.

Maintenance of calcium homeostasis is of crucial importance in the proper functioning of the immune system and inflammatory responses, such as responsiveness of T and B lymphocytes, differentiation of T helper cells into Th1 and Th2 subsets and secretion of proinflammatory cytokines. Activities of LTCC and elevations in intracellular calcium level are the central element in the activation of brain immune cells.

LTCC are also expressed in endothelial and smooth muscle cells that line blood vessels, where their activities are closely involved cerebral blood flow and maintenance of blood brain barrier, especially in the developing brain. The same is true of the cells lining the gastrointestinal tract, in which LTCC and cellular levels of calcium play an important role in gut inflammation and permeability, gut motility and gastric acid secretion. In addition, LTCC are expressed in pancreatic beta cells, where their activities govern many aspects of pancreatic function, including digestive enzymes and insulin production and secretion.

Calcium influx through LTCC plays a crucial role in mitochondrial calcium overload and downstream mitochondrial and cellular dysfunctions, and elevation of intracellular calcium level is responsible for activation of ROS-generating enzymes and formation of free radicals by the mitochondria.

Timothy syndrome is a multisystem disorder in which a mutation in a gene that encodes Ca(V)1.2 L-type calcium channel leads to loss of channel inactivation and subsequent intracellular calcium overload in various cell types. Such disturbances in calcium homeostasis are thought to underlie the multiorgan dysfunction observed in this disorder, which includes congenital heart disease, immune deficiency, irregular sleep patterns, hypoglycemia, cognitive abnormalities, and autism [15454078].

Autism, or Autism Spectrum Disorders (ASD), is a group of neurodevelopmental disorders that manifest at an early age and is characterised by impairments in social interaction, communication, interests, imagination and activities. Apart from neurobehavioural symptoms, ASD individuals frequently present with impairments in areas such as motor function and coordination, sensitivities and abnormalities in visual and auditory processing, various gastrointestinal symptoms, and immune dysfunction. As autism is a highly heterogenous disorder, the symptoms can vary greatly in each affected individual.
Numerous findings in recent years point to underlying biological abnormalities in autism, including irregularities in neurotransmitter systems, cholesterol metabolism, mitochondrial enzyme activities, and levels and secretion rhythms of hormones; decreased cerebral blood flow and increased cerebral water content; elevated markers of oxidative stress; altered intestinal microflora; and intestinal damage and inflammation. In addition to the active, ongoing inflammation in the gastrointestinal tract and the CNS in autism, results of numerous studies point to an abnormality of the immune function such as the absence of adaptive immune system/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 ratio.

It has been suggested in the past that disturbances in calcium signalling pathways may be the underlying molecular cause of autism [17275285]. Furthermore, a recent postmortem study 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).

This paper further explores the potential role of dysfunctional calcium homeostasis, and in particular the functional disturbances of voltage gated calcium channels, that could lead to pathologies of autism. Various factors that are capable of disturbing the functioning of VGCC in critical stages of human development are discussed. Particular attention is given to the role of chemokine receptors as modulators of calcium signalling and possible implications of these events in etiology of autism.

In addition, the different ways in which sex hormones influence functioning of VGCC are proposed to be the reason for greater prevalence of autism in males than females.
Calcium homeostasis in the Central Nervous System – implications for brain development and autism

Mechanisms related to calcium homeostasis that influence neuronal growth, branching, differentiation, maturation, motility and structural organisation in developing brain are reviewed in this section, together with their possible role in the etiology of 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)

* 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 neurogliai activation and neuroinflammation (see Immunity/Inflammation).

* 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 (see BBB).

* reduced blood flow to parts of the brain (see BBB)

* 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, 9619192, 18435417, 9758336, 15749244, 16819561, 16214373, 14519452, 16924017]

Intracellular calcium homeostasis is essential for neuronal development and function and calcium influx through voltage gated calcium channels (VGCC) regulates numerous processes in the central nervous system (CNS), including neuronal growth, differentiation, motility and excitability, secretion of neurotransmitters and hormones, synaptic plasticity, neurotoxicity and neuronal gene expression. Regulation of calcium entry through VGCC is also of major importance in sensory processing and motor function. Because of the critical role of calcium channels in signalling processes, disruption of their function can lead to profound disturbances in the structure and functioning of the nervous system. Elevated levels of intracellular calcium are involved in neurodegenerative mechanisms of the brain tissue and neurological disorders can be caused by mutations in genes encoding calcium channel subunits. There are currently several known human and mouse channelopathies of the CNS, including a recessive retinal disorder, X–linked congenital stationary night blindness, familial hemiplegic migraine, episodic ataxia type 2, and spinocerebellar ataxia. At present there are two known calcium channel genetic mutations directly linked to autism (see Genetic–Factors). Murine recessive neurological disorders as results of mutations in genes encoding calcium channels include the tottering, leaner, and rocker phenotypes with ataxia and absence epilepsy, and the rolling Nagoya phenotype with ataxia without seizures.

Ion entry into neurons occurs either through receptor–operated channels, for example GABA and NMDA channels, or through voltage–gated ion channels. Although it is recognised that calcium entry through both types of channels, as well as calcium released from internal stores [15709700]
may be important in the etiology of psychiatric disorders, this review will mainly focus on the role of voltage gated calcium channels, and L-type calcium channels (LTCC) in particular.

LTCC are localized on nerve terminals in the pre and postsynaptic parts, as well as on cell bodies. Although many cells throughout the body express LTCC, their density is higher in the brain, especially during both development and aging. Apart from neurons, calcium entry through LTCC is a major event in many processes in both microglia and astrocytes, the supporting cells of the CNS.

**Calcium homeostasis in developing brain**

Neuronal cell development is controlled by a tightly organised and regulated sequence of events that include cellular proliferation, differentiation, migration and maturation. Signalling by calcium ions plays a central role in these events.

**Age-dependant changes in calcium signalling**

VGCC are highly expressed during development and their function is critical for developing neurons. In postnatally developing brain, a transitional period for still developing neurons, there appears to be a critical window in development in which disturbances in calcium homeostasis may have significant consequences [16921238, 10493768].

In vitro neuronal cultures have exhibited great differences in sensitivity to changes in levels of intracellular calcium, depending on the exact stage of development. These age-dependent changes in functioning of VGCC have so far been linked to the onset of several developmental events, including neuronal differentiation [7515527], neurite outgrowth and synaptogenesis [7790927, 7965045]. An age-dependent role of LTCC in developmental regulation of transmitter phenotype in neurons has also been demonstrated, whereas the expression of tyrosine hydroxylase (TH), a dopaminergic marker, in developing neurons was shown to be dependent on the activities of LTCC [9437025] (see Neurotransmitters).

**Neuronal gene expression**

A transcription factor is a protein that acts as a regulator of gene expression. CREB (cAMP response element--binding) proteins are transcription factors which bind to cAMP response elements in DNA and thereby increase or decrease the transcription of certain genes. CREB has been widely studied due to its role in diverse functions such as circadian rhythms, drug addiction and inflammatory pathways. Both CREB and several transcriptional regulators have been linked to epigenetic factors involved in cognitive and behavioural developmental disorders [15721740]. CREB deficient mice for example were shown to exhibit less active and exploratory behaviours in novel environments, as well as memory deficits in spatial learning and fear conditioning [15233759, 15805310].

One of the ways in which calcium channels influence neuronal and many other activities is via signaling pathways that control gene expression. This involves regulation of various transcription factors, including CREB. Calcium entry specifically through LTCC is particularly important for transcriptional responses in neurons, muscle, pancreatic beta cells and osteoblasts. Through its stimulation of CREB nuclear calcium may modulate the expression of numerous genes including neurotransmitter receptors and transmembrane and scaffolding proteins, with the involvement of most having been implicated in autism (see Neurotransmitters and Genetic–Factors). LTCC in brain have been implicated in mediating many long term changes in neuronal activity, some having behavioural and cognitive modulation as their end results. This importance of LTCC function in gene expression has been observed in many diverse neurons, including those found in the hippocampus, cortex, striatum, retina, dorsal root ganglia and cerebellum. Early developing Purkinje neurons prior to the stage of dendritic development express a somatic calcium signaling pathway that communicates information from the cell membrane to the cytosol and nucleus [16035195] (see next).
Amongst other effects, opening of LTCC leads to CREB induced expression of brain derived neurotrophic factor (BDNF) and neuronal nitric oxide synthase (nNOS) [11572963, 14604759]. nNOS activity regulates the production of nitric oxide, excessive levels of which can be damaging to neurons, causing oxidative stress and cell death. Calcium channel mutant mice that display similarities with human neurological conditions, including autism, all exhibit varying degrees of cerebellar dysfunction and neuronal cell death, thought to be at least partly due to abnormal nNOS [12834873].

With regards to BDNF, its levels and levels of BDNF autoantibodies are known to be elevated in brains of individuals with autism, with one study observing them to be three times higher than controls, with on the other hand significantly reduced blood levels in adults with autism [16181614, 11431227, 16876305]. Excessive activation of LTCC causes granule cells to express BDNF, the release of which stimulates tyrosine kinase receptors (TrkB) to induce axonal branching, which may establish hyperexcitable dentate circuits implicated in epilepsy [15317847]. Exploring TrkB partial agonists as a possible treatment option for autism has been suggested [16023301]. The mechanism of calcium and BDNF signalling also plays a role in establishing granule cell synaptic transmission, including levels of expression of NMDA receptors, during cerebellar development [16221864].

Apart from BDNF, its neurotrophin family includes the growth factors Nerve Growth Factor (NGF), neurotrophin 3 (NT-3), and neurotrophin 4 (NT-4), some of which were also found to be elevated in autism in several studies [11357950, 16289943]. The same studies observed raised levels of neuropeptide vasoactive intestinal peptide (VIP) compared to controls. The expression level of VIP is influenced by calcium influx through LTCC, possibly through similar mechanisms [15197736]. On the other hand VIP is able to influence VGCC conductance through its known interaction with G-protein-coupled receptors [8772132, 15109935].

In addition, significant elevations of neuropeptide vasopressin (AVP), with concurrent reductions in levels of aepenin, a neuropeptide that could counteract AVP action, have been observed in autism. Again the involvement of raised calcium levels and CREB activities has been suggested in the expression of vasopressin gene [3607454, 9389510].

Possible involvement of Homer and Shank protein complexes in the LTCC activation of CREB has been suggested [15689539, 12716953], as localized calcium responses, regulated by interactions with PDZ domain proteins, are deemed necessary for this activation. It should be mentioned that loss of the SHANK3/PROSAP2 gene has been proposed to be responsible for the main neurological developmental deficits observed in 22q13 deletion syndrome, characterised by delays in speech and motor development [16284256] (see Genetic Factors). Chromosomal deletions of SHANK3 have recently been identified in a small number of individuals with autism.

With reference to CREB-related activities possibly being relevant in the etiology of autism, it should be added that sex hormone estradiol has been noted to regulate CREB activity via its direct and/or indirect effect on LTCC, and that considerable overlap between behaviors and processes reliant on CREB and those that are influenced by estradiol has been noted [15901789] (see Gender Differences).

A reduced MET gene expression has been implicated in autism susceptibility. A study analysis of the gene encoding the pleiotropic MET receptor tyrosine kinase observed a decrease in the promoter activity of the gene and altered binding of specific transcription factor complexes in autism sample [17053076]. MET signaling plays a role in neuronal growth and maturation as well as in the immune function and gastrointestinal repair, two areas with frequently reported medical complications in autism. The expression of tyrosine kinase receptors is linked to calcium signalling pathways, and sudden changes in the levels of intercellular calcium from both external and internal sources results in changes in levels of MET tyrosine phosphorylation. This regulatory effect of calcium is mediated through calcium-linked proteins and enzymes [1651934, 2111905, 2005882].

It has been suggested that an important subset of developing hippocampal interneurons expressing inhibitory GABA and GAD enzymes express LTCC and that these channels likely regulate the development of these interneurons [16154277, 11085875], as well as the expression levels of GAD and GABA (see Neurotransmitters).
Increasing evidence suggests that the observed down-regulation of Reelin mRNA in neurological disorders may be caused by the dysfunction of epigenetic regulatory pathways in these interneurons [17065238]. Reelin is a protein that is found mainly in the brain and that acts on migrating neuronal precursors and controls correct cell positioning in several areas of the brain. It is secreted by Cajal–Retzius cells and by the external granule cell layer in the cerebellum and its release rate depends solely on its synthesis rate. Abnormalities in the expression levels of Reelin protein and mRNA, as well as those of Reelin receptor VLDLR in frontal and cerebellar areas of autistic brains versus control subjects have been observed, implicating impaired Reelin signalling in autism [15820235]. Several linkage studies have so far failed to establish a firm genetic basis of this abnormality [15048647, 15048648].

It merits a mention in this context that the level of Reelin can be affected significantly following exposure to x-radiation [10744063]. Whether the effect of x-radiation on calcium channel conductance could be one likely mechanism behind this effect remains to be established [9096258]. Of equal interest is the observation that in rodents prenatal viral infection leads to significant reduction in production of Reelin [10208446] (see Viruses).

In addition to CREB, LTCC activate a number of other transcription factors such as NFAT, MEF-2, and SRF. The nuclear factor of activated T-cells (NFATc) was originally characterized in the immune system, but is now known to play an important role in brain function as well [link].

Another suggested mechanism for this privileged role of calcium channels in epigenetic pathways is the role of the calcium channel-associated transcription regulator (CCAT). CCAT binds to a nuclear protein and in this way regulates the expression of a wide variety of genes involved in neuronal signaling and excitability. The nuclear localization of CCAT is regulated both developmentally and by changes in intracellular calcium. If confirmed, this mechanism would provide a more direct way in which VGCC activate gene transcription in excitable cells [17081980].

**Purkinje neurons**

Purkinje cells are a class of GABAergic neurons located in the cerebellar cortex. These cells exhibit a highly intricate dendritic arbour, with a large number of dendritic spines. These cells are of central importance for bodily functions of balance and coordination. Cerebellar abiotrophy is a condition affecting some animals in which Purkinje cells begin to atrophy shortly after birth, and this often results in symptoms such as ataxia, intention tremors, hyperreactivity, stiff or high-stepping gait, apparent lack of awareness of where the feet are, and a general inability to determine space and distance. A similar condition known as cerebellar hypoplasia occurs when Purkinje cells either fail to develop or die prenatally.

The proliferation and survival of GABAergic neurons, including Purkinje neurons, in developing brain seems to be dependent on highly regulated calcium influx through VGCC. Increasing or decreasing calcium currents through these channels was observed to have profound effects on survival of cell cultures. This rate of dependence and survival seems to be linked to the exact stage of development (see above) [10366697]. As an illustration, the age-dependent effect of ethanol, a toxic environmental factor, on developing Purkinje neurons is well known, with ethanol being able induce to mitochondrial damage and ultimately cell death [12204202]. These effects are likely due to ethanol-induced changes in VGCC function and altered calcium signalling [16555300](see Mitochondria).

Young and undeveloped Purkinje neurons without dendrites in culture express only the high-threshold calcium current, which increases approximately by half in amplitude during development, thus indicating the importance of calcium conductances in development and maturation of early Purkinje neurons [1377238]. One prominent function of calcium entry through LTCC in these neurons is that this signalling pathway appears to convey developmental cues directly to the nucleus, thus influencing activation of gene transcription factors, CREB in particular (see above), and expression of cellular proteins. This effect can be additionally amplified by release of calcium from intercellular stores [16035195, 16555300, 11007898].

**Neuronal differentiation, growth, branching, migration and structural organisation**
Calcium signaling regulates both axonal and dendritic branching in most types of developing neurons, including Purkinje neurons [11248350]. Some of the mechanism of this effect is via abovementioned calcium-dependent activation of CREB, its effects on cytoskeleton and its regulation of the expression levels of neurotransmitters and neurotrophins and activation of protein tyrosine kinases [15581694, 15882639, 8845164]. Calcium regulation of neurite growth and growth cone motility is a process that is dependent on activation G–proteins and is sensitive to pertussis toxin treatment.

Optimum levels of calcium influx promote normal dendritic and axonal elongation and growth cone movements, which are involved in neuronal directional pathfinding and target recognition. These activities are essential for assembly of functional circuits within the developing nervous system and for regeneration following damage. Changes in levels of intercellular calcium and its way of entry into the cells thus can have profound effects on the structure and function of these neuronal networks [3121806]. Calcium transients regulate growth cone advance by direct effects on the growth cone. These transients are mediated primarily by LTCC and silencing them with channel blockers can in some circumstances promote axon outgrowth [12574421]. Several factors that are though to influence neurite outgrowth, for example serotonin and acetylcholinesterase, are suggested to exert that influence through activities of LTCC [12031351, 2376732, 10437116]. Brain serotonin levels play an important role in developing brain and impairments of serotonin metabolism have been implicated in autism. In addition, a recent study has described maternal serotonin levels as being of central importance for developing fetal brain (see Maternal_Factors). In addition, significant preturbations in brain levels of tryptophan and/or serotonin and its receptors have been recorded in some viral infections [8158981, 3509812] (see also Viruses). 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, 11494406, 12401168]. On the other hand, calcium signals and functioning of VGCC play an important role in serotonin metabolism and secretion, and in the regulation of serotonin receptors expression and function (see Neurotransmitters).

It has been suggested that calcium signals through LTCC influence differentiation of neural stem/progenitor cells (NSC). A study looking at NSC derived from the brain cortex of postnatal mice observed that their differentiation is strongly correlated with the expression of LTCC, and that influx of calcium ions through these channels plays a key role in promoting neuronal differentiation [1094458, 16519658]. In addition, calcium transients also play a central role in controlling migration and organisation of both neuronal and non–neuronal cells in the developing CNS [15820385, 16720042, 16029198, 15712206]. In vitro, neurons migrate in association with nonneuronal cells to form cellular aggregates. Changes in those cell complexes in cultured embryonic chick ciliary ganglion were observed in response to treatments that increased or decreased intracellular calcium concentration. Application of thimerosal, a compound that stimulates calcium mobilization from internal stores, increased the amplitude of spontaneous nonneuronal oscillations and the area of migrating nonneuronal cells as well as the velocity of the neuronal–nonneuronal cell complex [16720042].

In mouse ‘weaver’ phenotype the genetic mutation impairs migration of the cerebellar granular neurons and induces neuronal death during the first two weeks of postnatal life. Upregulation of calcium channels was found to contribute to the migration deficiency of these neurons. Loss of these neurons could be attenuated by application of LTCC blockers [8707831]. Increases in intracellular calcium levels via upregulation of VGCC activates calcium–calmodulin–dependent protein kinase (CaMk) and calcineurin phosphatase (CaN), which play an important role in development and synaptic organization of granule cells during early postnatal period [16793900].

**Neuronal apoptosis**

LTCC involvement in neuronal apoptosis (cell death) is probably at least partly due to mitochondrial injury induced by excessive calcium influx and ROS (see Mitochondria and Oxidative Stress). Several LTCC antagonists are able to attenuate cell injury and death in culture, as being...
induced either by some pathogens, including Amyloid beta protein implicated in etiology of Alzheimer's disease [15303126, 16321794, 15006551, 10964602] (see Related Disorders).

**Synapse formation and synaptic plasticity**

It has been hypothesised that autism and related symptoms could in part be a result of disruption of synaptic plasticity in developing brain [15362161]. Synaptic plasticity is the ability of the synapses between two neurons to change in strength. One of the mechanisms underlying synaptic plasticity involves regulation of gene transcription and changes in the levels of key proteins at synapses [9437025]. Several recent studies have established a role of LTCC in long-term potentiation (LTP), a long lasting enhancement in efficacy of the synapses between the neurons, thought to be the cellular basis of learning and memory [16251435]. One good example is cpg15, a gene that encodes a membrane-bound ligand that regulates neurite growth and synaptic maturation, and whose expression level is thought to be at least partly influenced through LTCC activation of CREB [14664806]. LTCC are crucially involved in regulation of synapses of auditory inner hair cells, which includes regulation of the expression of potassium channels on those synapses [16828974] (see Motor/Sensory).

**Calcium homeostasis in neuroglia**

Glia cells are cells that provide support and nutrition to neurons. Astrocytes, the largest and most abundantly expressed glial cells, form connective tissue of the brain and carry out various functions, including induction of neuronal growth and differentiation, participation in maintenance of blood brain barrier and cerebral blood flow, regulation of ion concentration in the extracellular space and modulation of synaptic transmission. Astrocytes accumulate in areas where neurons have been damaged (gliosis). Microglia are macrophages that have immunoprotective role in the brain and play an important role in inflammatory responses.

Changes in intracellular calcium levels are an important signal for communication between glial cells and neurons, and recent evidence points to voltage gated calcium channels as playing an important regulatory role in these processes. For example transient increases in calcium levels in astrocytes either from external sources or from internal stores can result in release of glutamate and modulation of synaptic transmission in surrounding neurons [14966867, 12555202]. Furthermore, reactive gliosis as well as glial cell injury and death is thought to be mediated by upregulation of VGCC [9502793, 9736645]. Astroglial release of proteins which enhance neuronal survival and induce neuronal growth and differentiation can be blocked by calcium antagonists and mimicked by Bay K 8644, a calcium channel agonist, indicating the importance of calcium homeostasis in these events [1397177].

For importance of calcium homeostasis in regulation of cerebral blood flow as well as maintenance of blood brain barrier by astrocytes see BBB.

Microglia play an important role in CNS inflammatory responses, and its migratory and secretory responses can be modulated by increases of calcium via LTCC. Several proteins and lipopolysaccharides are known to be able to exert such influence, either directly or through activation of chemokine receptors (see Immune/Inflammation) [10858625, 9914452, 12805281]. Similar mechanism of rises in calcium levels following activation of chemokine receptors has been observed in oligodendrocytes, whose main function is to myelinate axons [16095689]. Stimulation of CXCR4 receptors and subsequent elevation of calcium is in these neuroglial cells is a G protein-linked event [16837851]. It may be of interest in this context that an experimental mouse model of multiple sclerosis was successfully treated with calcium antagonists bepridil and nitrendipine [15296830]. Furthermore, it has been proposed that oligodendrocytes, alongside astrocytes, play an important role in regulating potassium levels (see Epilepsy/Seizures).
**Neurotransmitters in autism and role of calcium signalling**

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 major histocompatibility complex (MHC) types associated with autism [8904735].

Calcium influx through VGCCs is a key step in secretion of neurotransmitters, for example serotonin [16047543]. Due to vesicle priming in neuronal exocytosis, the influx of calcium ions is all that is needed to trigger nearly instantaneous neurotransmitter release in neurons [12043844]. Moreover, some findings indicate that its excessive entry through LTCC during early development may to alter neuronal response properties at later ages [9437025]. Especially in developing brain modulation of neurotransmitter release by dihydropyridine-sensitive calcium channels involves tyrosine phosphorylation. As the neurons develop a network of neurites, both tyrosine phosphorylation and LTCC activity seem to decrease [9987031, 11226706] (see Brain). This transmitter–secretion effect of LTCC is G-protein-linked and sensitive to pertussis toxin treatment [8994064]. Influx of calcium through N-type VGCC directly stimulates dopamine release and this effect can be attenuated by chalcium channels blockers [11769325, 15272204]. The involvement of LTCC-linked IP3-sensitive intercellular stores in the calcium–triggered release of dopamine and acetylcholine has also been observed [14657041]. Secretion of beta–endorphin, whose levels are significantly elevated in autism, is also triggered by calcium influx into the cell and can be lowered in vitro by applying calcium channel blockers [2428932, 10371405].

Following the findings of significant modifications of catecholamine metabolites in autism it may be worth mentioning that the activities of Catechol–O–methyl transferase (COMT), an enzyme involved in the breakdown of the catecholamine neurotransmitters, are inhibited by raised calcium levels in tissue [12170607]. Additionally, a small pilot study examining administration of tetrahydrobiopterin (R-BH4), a cofactor for tyrosine hydroxylases in the pathway of catecholamines and serotonin, reported amelioration of several autistic traits in study subjects. Decreased dopamine D2 receptor binding was also reported (see below) [9236697]. Tyrosine hydroxylase (TH) is an enzyme of central importance in catecholamine biosynthesis and the expression level of its gene is controlled by several calcium signalling pathways, most importantly LTCC–regulated CREB (see Brain) [15001085, 9645965]. In so called Tottering mice, an animal model with inherited mutation in calcium channels, the increased density of LTCC in the brain is followed by abnormal regulation of tyrosine hydroxylase. In vivo chronic nimodipine treatment was shown to significantly reduce the expression of TH mRNA in these mice [14715436].

In vivo application of calcium channel agonist and antagonists points to a possible role played by calcium inward currents in synthesis and metabolism of dopamine and serotonin in brain, with different effect observed in specific areas of rodent brain [7683338, 2431107, 7545305]

Abnormal stimulation of dopamine or serotonin receptors have been hypotesised as able to lead to the types of neuroanatomical changes observed in autism, schizophrenia and bipolar disorder. Of relevance is the close interplay and interdependence of dopamine receptors and VGCCs,
especially the effect of D1 receptor activation on LTCC function and CREB–influenced gene expression. It has been observed that activation of dopamine D1 receptors alters the properties of LTCC blockers and turns them into facilitators of calcium influx. In other words in D1 receptor–stimulated neurons these agents, instead of blocking calcium, actually promote its entry, which leads to the activation of signalling pathways and CREB phosphorylation. [15530653, 14622123]. (see Brain). These same mechanisms have been observed in the effects of psychostimulants on gene expression [16724157].

In addition, D2 and D4 dopamine receptors, together with muscarinic receptors (especially M1), are also able to modulate one of the subtypes of LTCC. These effects are likely controlled by pertussis toxin sensitive G–proteins and linked to postsynaptic density proteins, notably Shank [15689540, 12496094, 7477916, 10437116, 9000430, 15615835].

Of possible relevance in this context is also the involvement and influence of opioid receptors on these mechanisms, and the observation that Naltrexone is able to modulate the functioning of D2 receptors, in a dose dependent manner.

Brain serotonin level is suspected to play an important role in developing brain and impairments of serotonin metabolism have been implicated in autism. Maternal serotonin levels have been suggested as being of central importance for developing fetal brain (see Maternal). Reduced brain levels of tryptophan and/or serotonin in the brain and its receptors have been recorded in some viral infections (see Viruses). 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, 11494406, 12401168]. Calcium influx through LTCC may play an important role in the regulation of the serotonin 5-HT2A receptor expression levels and function [11474845]. Another pathway that influences the expression of these receptors is linked to activation of PKC [9928249, 11299321]. It has been observed that activities and expression of 5-HT2A and several other serotonin receptors are also tightly linked to the levels of cholesterol as well as caveolin Cav–1 (see Membranes and Smith–Lemli–Opitz Syndrome) [17064686, 15157621, 15190056]. Levels of membrane cholesterol have also been suggested to play a role in activities of serotonin transporter (SERT), responsible for the reuptake of serotonin [11523992].

Presynaptic 5-HT3 receptors are permeable to calcium and modulate neurotransmitter release. Interestingly, calcium entry through 5-HT3 receptors can, depending on receptor location, be blocked by LTCC channel antagonists [9489730, 15541891].

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 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. This mechanism is linked to activation and function of both LTCC and intracellular calcium receptors [11157063, 12065669, 11498514]. On the other hand, there is now ample evidence that altering calcium dynamics can modulate neuronal nAChR function [7542542, 9415721, 12915265]. 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]. Gamma-aminobutyric acid GABA is the chief inhibitory neurotransmitter in the central nervous system. Glutamic acid decarboxylase (GAD) is the enzyme responsible for conversion of excitatory neurotransmitter glutamate to GABA in the brain, and its activity is regulated by calcium homeostasis – it has been demonstrated that the activity of GAD depends on the strict balance of extracellular and intracellular levels of calcium, as well as between the free and stored calcium in the cell [6856025, 12603819, 10366697, 12603819]. In addition, the expression levels of mRNA of genes encoding for GAD and GABA appear to be regulated by calcium transients in developing neurons [11085875, 16154277] (also see Brain)
Also worth noting is that glutamate has been implicated in epileptic seizures: “Microinjection of glutamic acid into neurons produces spontaneous depolarisations … and this firing pattern is similar to what is known as paroxysmal depolarising shift in epileptic attacks. This change in the resting membrane potential at seizure foci could cause spontaneous opening of voltage activated calcium channels, leading to glutamic acid release and further depolarization...”. There is growing evidence that apart from NMDA receptors, LTCC also play a role in glutamate excitotoxicity [10493768].

In rat dopaminergic neurons secretion of excitatory amino acids aspartate and glutamate was found to be directly regulated by activities of L-type calcium channels, and it was suggested by the authors that the drugs that modulate presynaptic LTCC may show to be of use in neurological and psychiatric disorders that involve the dopamine system [9712641].

The coupling of cholinergic, dopamine and serotonin receptors to calcium channels and their sensitivity to cellular calcium dynamics is proposed to be one of the reasons for the observed abnormalities of these systems in autism. Disturbed calcium homeostasis could also be one likely mechanisms behind the abnormal secretion rhythms of various neurotransmitters as well as the lowered activities of GAD and abnormal levels of glutamate in autism.
Autism, hormonal metabolism and calcium signalling

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]. Overreaction 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-acetyltransferase 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].

Calcium influx through voltage gated calcium channels is directly involved in both neurotransmitter and hormone secretion. In newborn mice the relative dominance of LTCC over other types of calcium channels has been observed [14724188]. LTCC are present on different pituitary cells and their activity is in part modulated by sex steroids [2461851] (see also Gender Differences). Hormonal secretion evoked by various agents is mediated via calcium influx through LTCC [9514161, 15500542, 8677013, 1649931].

Calcium signalling plays a central role in regulation of melatonin biosynthesis, mostly through activities related to phosphorylation of the transcription factor CREB [9618900] (see Brain for details on LTCC–CREB). Changes in conductance of LTCC and intracellular calcium oscillations have dramatic effects on melatonin levels [10820209] (see also Neurotransmitters– serotonin).

Calcium signalling though LTCC plays an important role in the release of insulin and regulation of the expression of its gene (via CREB mediated transcription). Significantly increased amounts of calcium in the cells cause release of previously synthesised insulin, stored in secretory vesicles (see Gastrointestinal). Of possible relevance is the observation that both low and elevated or sustained levels of intracellular calcium impair insulin–stimulated glucose uptake [2551647, 3312189].

Thus calcium appears to required for glucose utilization and plays an essential role in the stimulatory effect of insulin on leptin secretion. Hoever, excess calcium disrupts leptin secretion by interfering with metabolic events that are independent from glucose uptake [15331383].

Oxytocin is a neuropeptide hormone that also acts as a neurotransmitter in the brain and together with vasopressin, another posterior pituitary hormone, has a role in regulation of social bonding and behaviors in mammals such as mating, pair-bond formation, maternal and parenting
behavior, and attachment [16884725]. Rodents lacking the oxytocin or vasopressin gene or those lacking the vasopressin V1a receptor show significant deficits in social behaviour and social recognition [15749248]. It has therefore been suggested that deficiencies in oxytocin pathways in the brain might be a feature of autism. Parallel to the animal model studies actual alterations in endocrine oxytocin system have been observed in children with autism [11690596].

It may be of relevance in this context that V1a vasopressin receptor is a G-protein coupled receptor functionally tightly linked to LTCC. This coupling is sensitive to pertussis toxin treatment [8913359]. When stimulated by an agonist (vasopressin) this receptor is able to induce a complex intracellular calcium signalling cascade for gene expression in astrocytes, eventually influencing CREB-mediated events and decreasing expression levels of several cytokines, notably interleukin-1beta and tumor necrosis factor-alpha. This V1 agonist-induced decrease of cytokine release from cortical astrocytes was also shown to be neuroprotective in cortical neurons [14999073]. In cultured cortical neurons, V1aR activation again influences the influx of extracellular calcium via regulation of activities of LTCC [11726244]. Oxytocin also seems to be able to regulate calcium currents via LTCC [7530160, 11757073]. On the other hand the opposite mechanism has been observed, whereas entry of calcium through LTCC influences release rate of vasopressin and oxytocin hormones [7957609]. Even more interesting and of possible relevance to autism is the observation that although the secretion of these two hormones seems to be induced by calcium influx at initial stages, the prolonged activation of LTCC results in decline in their secretion [2072100].
Motor and sensory disturbances in autism and role of calcium signalling

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 ideomotor dyspraxia, a difficulty with executing a known plan. Various areas of difficulty can include speech and language, fine motor control (e.g., handwriting or holding a 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.

During development and growth motor neurons express multiple calcium channels that are thought to be involved in their development. The importance of LTCC and ryanodine-sensitive calcium channels in particular has been observed [16324742, 9758236]. Several types of ataxia in humans are results in mutations in genes encoding for calcium channels [16100538]. Rodent models with mutations in genes that encode for calcium channels exhibit various forms of motor abnormalities, including ataxia and dystonia, as well as lower body weight [9882694, 12890513]. Administration of calcium channel agonist BAY K 8644 to wild-type mice results in similar motor dysfunctions and dystonia, which could be reversed by applying LTCC blockers [10830422]. In addition, mice with mutation in genes that encode for calcium binding proteins also exhibit significant deficits in motor coordination as well as sensory processing, suggesting the importance of intracellular calcium buffering and regulation in these functions [9037080, 12716955, 10220453]. Decreased expression of calcium binding proteins has also been suggested to be behind impaired motor function following hypobaric hypoxia [16169666] (see Treatments).

With regards to role of calcium homeostasis in auditory processing, LTCC located in inner ear hair cells are essential for auditory processing in mammals, and are involved in development of auditory system [15115817, 16828974, 14645476, 16567618, 15158080].

Animal models have shown that mice lacking a gene that encodes one subunit of LTCC exhibit reduced auditory evoked behavioral responses [12890513, 15283975], whereas mice treated with calcium channel agonist BAY K 8644 exhibit, alongside deficits in motor activity and coordination, a significantly increased sensitivity to auditory stimulation, which can be reversed by dihydropyridine calcium channel antagonist nifedipine [2581145]. As with abovementioned motor neurons, again the importance of calcium buffering and calcium binding protein expression has been observed in auditory outer hair cells, in which they are thought to play a developmental role and in which cellular calcium overload as a result of acoustic overstimulation can be amplified in the absence of those proteins [16120789, 8867285].

Apart from motor and auditory abnormalities, another phenomenon observed following administration of calcium channel agonists to mice is the emergence of self-biting behaviour, which could be inhibited by pretreating the mice with dihydropyridine LTCC antagonists [10611367].

Development and refinement of retinal pathways is partially dependent on function of voltage gated calcium channels and calcium fluxes into, and within the cell. LTCC in particular are expressed in many neurons linked to retinal pathways during development [12101036, 2838315].
In animal models, development of the visual pathways is disrupted in mice with a disruption of a calcium channel subunit genes [11745616, 17033974].

In humans, several inherited retinal disorders have been associated with mutations in genes encoding for voltage gated calcium channels. For example, a mutation in a calcium channel gene Cacna1f that leads to retinal disorder and visual impairments has been observed in a family in New Zealand. Although female members of the family display visual impairments, the symptoms are more severe, and include abnormal colour vision, a symptom that is common in autism, in male family members. Five of the affected males exhibit intellectual disability, with autism being present in three of those five individuals [15807819]. Perception of colour is linked to retinal cone cells, and calcium dynamics and functioning of LTCC plays a central role in those cells [12161344].

Animal studies have pointed to the role played by LTCC in fear conditioning and implications of these mechanisms in the treatment of anxiety and in emotional learning and plasticity [12724155].

Several calcium channels expressed in different types of neurons and at different locations have been implicated in pain perception and signalling [12832498, 1425934, 11520183, 15843607].
Disturbances in calcium signalling and implications for cerebral blood flow, edema and Blood Brain Barrier 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] 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). 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.

For many vessels, including cerebral arteries, calcium entry through LTCC constitutes the main fraction of contractile calcium. This is particularly true for immature cerebral arteries, which are totally dependent on calcium influx through LTCC channels for contraction, due to relative lack of intercellular calcium stores, and in which the expression of these channels is twice as high as in the adult arteries [11742831]. One of possible mechanisms through which excessive calcium influx via LTCC could be causing restricted blood flow is through its effect on synthesis rate of endothelin–1, a potent vasoconstrictor in microvascular endothelial cells [12388093]. Following these findings, a scenario can be suggested in which disturbances in the functioning of LTCC can easily lead to vasoconstriction and decreased cerebral blood flow, as observed in autism.

In an experimental animal model of hydrocephalus and chronic cerebral ischemia, protective effect against declines in motor and cognitive behavior exerted by nimodipine, a LTCC blocker, was thought to be most likely based on improved blood flow [11354411]. Several other calcium channel antagonists have shown various degrees of neuroprotection through improvement of cerebral blood circulation [9877076].

In relation to increased water content in the brain (brain edema), one critical event in its development is breakdown of tight endothelial junctions which make up the blood–brain barrier (BBB), which allows fluid to penetrate into brain. Calcium plays a major role in endothelial junctions, whose function is necessary for the barrier characteristics of cerebral microvessels. G–proteins and several calcium linked proteins and enzymes also seem to be closely involved in junction formation and maintenance [12053015, 1920385]. Calcium ions could therefore alter BBB junction integrity through various signalling cascades, as well as through direct interaction with junction proteins. Regulation of extracellular and intracellular calcium levels seems to be critical in the normal functioning of the BBB.

Several proinflammatory mediators, including various cytokines and chemokines, have direct and indirect effects on the BBB leading to BBB disruption [16671502]. Humal endothelial cells express functional chemokine receptors [9461627, 10479649] that can influence calcium homeostasis via LTCC, leading to disruption of endothelial cellular function. In particular the expression of chemokine receptor CCR2 in human cerebral endothelial cells, with its important role in regulating brain endothelial permeability, has been uncovered recently [16192992]. Its ligand, monocyte chemoattractant protein–1 (MCP–1) may cause permeability changes human vascular endothelium cells, possibly through reduced tight junctions of vascular endothelium cells [17098977]. Of particular interest should be also the expression of CCR2 receptors in fetal astrocytes, which together with endothelial cells form BBB, and the interactions of these receptors with MCP–1 and calcium signalling [12271471, 15689955]. Disruption of chemokine receptor CCR2 abolished both CNS inflammation and encephalopathy in a murine study [12486156] (also see Immunity–Inflammation and Viruses for more details on chemokine receptors and calcium signalling and findings of excessive levels of MCP–1 in brain and CSF in autism).
These chemokine receptors can be activated by viral proteins, for example the chemokine-like protein from human herpesvirus 6 was found to cause calcium mobilization through the CCR2 receptor. It has been suggested that this protein during reactivation of the virus could perhaps be involved in the pathogenesis of the CCR2-dependent disease, multiple sclerosis [12554737]. Infection of mouse epithelial cells with the influenza A-type virus strain strongly induced the expression of CCR2 and CCR5 receptors, followed by a strong monocyte migration [16849492]. In this context it should be mentioned that prenatal influenza infection has been implicated in the etiology of autism (see Viruses).

In the case of the widely studied HIV-1 virus, while its Tat protein is thought to induce polarization of CCR2 receptors in astrocytes [15578658], its protein gp120 has been found to compromises blood–brain barrier integrity by directly altering expression of tight junction proteins in brain microvascular endothelial cells. The mechanism of action behind this effect seems to be linked to its activation of chemokine receptors CCR5, protein kinase C (PKC) pathways and subsequent release of calcium from intercellular stores [16685256].

While for both HIV and HCMV, binding of viral glycoproteins to the cell surface is sufficient to induce a calcium response, herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) affect calcium signalling pathways in endothelial cells by triggering release of calcium from endoplasmic stores via IP3 receptor activation, with subsequent additional rises in calcium levels due to activation of IP3-linked voltage gated membrane channels [14568989].

It may be of interest in this context to note the observation concerning infection level of endothelial cells by human cytomegalovirus (HCMV) being influenced by level of PKC. In other words stimulation of this signalling pathway prior to infection results in an increase of infection by HCMV while its inhibition prevented virus replication in murine studies [9175259, 16033962]. Similar effects have been observed in respect to Epstein–Barr Virus (EBV) replication [2155183]. It should be noted that calcium is one of the activators of PKC.

In addition to the implications of chemokine–calcium signalling interactions in the endothelial and immune cells, similar events have been suggested to take place in neurons as well, whereas viral protein interference with chemokine receptors is able to influence downstream calcium–linked events, including activation of CREB and interference with cell–cycle proteins in neurons [11880151, 9826729, 12775414]. (see Brain and Viruses).

Apart from viruses, several toxic agents have been shown to directly interfere with calcium homeostasis in endothelium, thus contributing to their neurotoxic effects. For example exposure of cerebral vascular smooth muscle to methanol results in significant elevation in intercellular calcium. This methanol–induced cerebral vasospasm as a consequence of large rises in calcium levels is thought to play a central role in methanol–induced cerebral edema, brain hemorrhage, and cerebral and retinal infarcts, resulting in severe deficits in brain blood flow and disturbances of the CNS [10456574]. Similarly, many of the neurotoxic effects of lead are supposed to be related to its ability to dysregulate calcium signalling in cerebral arteries, leading to breakdown of BBB. Apart from its effects on the endothelial cells, another mechanism by which lead disrupts BBB is by damaging the astrocytes, which together with endothelium form a functional BBB. This damaging effect on astrocytes is also at least partly due to dysregulation of LTCC function by this heavy metal [1671748] (see Toxins). A similar mechanism has been hypothesised to be behind the neurotoxic effect of methylmercury, suggested to be secondary to astrocytic damage and disruption BBB brought about by the compound [15288515].

Relative to the function of astrocytes in maintenance of BBB and cerebral edema, the role of brain aquaporins in regulation of water homeostasis and the cerebro spinal fluid formation should be mentioned. Perturbed water flow via brain aquaporins has been implicated in many neurological diseases [15561410] and the involvement in the brain edema formation of several aquaporins that are abundantly expressed in astrocytes, at the blood brain barrier, has been reported recently. Calcium is known to play a role in expression of aquaporins and the involvement of LTCC in aquaporin functioning has been implicated in several studies [11353665] and calcium signalling and its effects on vasoconstriction by astrocytes is one of the mechanism for the regulation of cerebral blood flow [15356633]. Of interest are the increased levels of brain aquaporin 4 in autism [18435417].
Calcium overload therefore plays an important role in the occurrence and development of brain water edema. Treatment with nimodipine can dramatically reduce the damage of acute infectious brain edema induced by administration of pertussis toxin and thought to involve the opening of calcium channels in endothelial and neuronal cells. This effect is thought to be partially due to the reduction of the disruption of BBB by this calcium antagonist, although the opposite effect has been observed in acute brain damage, in which nimodipine treatment intensified edema formation [12659709, 9868080, 2118717, 3312189]. Benidipine, another calcium channel blocker, is also shown to restore endothelial function under particular circumstances [16172001].
Immunity and Inflammation

**Immune findings 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 matter, and in the cerebellum. A marked microglial and astroglial activation was also found, as well as the presence of an altered cytokine pattern, with macrophage chemoattractant protein (MCP)-1 and tumor growth factor–beta1 (TGF–beta1) being the most prevalent cytokines. There was also an accumulation of macrophages and monocytes, and a marked absence of lymphocytes and antibodies, pointing toward an innate neuroimmune activation with the absence of adaptive immune system/T cell activation. In addition, an enhanced proinflammatory cytokine profile was observed in the CSF, including once more a marked increase in MCP-1. These observations resemble findings in other neurological disorders in which elevations in cytokine levels is associated with the pathogenesis of neuroinflammation, neurotoxicity and neuronal injury and subsequent behavioural and cognitive impairments, for example HIV–associated dementia and multiple sclerosis [15288500, 11282546, 16875710, 9852582]. Another study examining brain tissue of found that proinflammatory cytokines TNF-alpha, IL-6 and GM-CSF, IFN-gamma and IL-8 were significantly increased in the brains of ASD patients compared with the controls. However the Th2 cytokines (IL-4, IL-5 and IL-10) showed no significant difference. The Th1/Th2 ratio was also significantly increased in ASD patients, suggesting localized brain inflammation and autoimmune pathology [19157572]. Further findings suggestive of autoimmune pathogenesis in a subgroup of patients revealed higher levels of antibodies directed against human cerebellar protein extracts [18706993], and high anti-nuclear antibody seropositivity in ASD patients compared to controls. In addition, Anti-nuclear antibody seropositivity 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 [19073846].

Another investigation into inflammatory markers in the brain tissue of patients with autisms 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].

Of some interest in this context may be the role of IL–6, a cytokine that is known to modulate cerebral function and enhance neurotoxicity through influencing Purkinje neuron physiology and electrical activity. Animal experiments illustrate that, during early pre and postnatal development, inflammatory cytokine challenge can induce various psychological, behavioral and cognitive impairments [17804539, 16147952, 9852582]. At the same time the expression of many cytokines, including MCP–1, in neurons and glial cells seems to be upregulated by increased intracellular calcium triggered by membrane depolarisation [11102468, 10943723] (see next section).

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 [18762240,16698940, 16360218]. It is of interest to note that, following increased levels of TGF–beta1 in brain
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-measles, 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).

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. It is very closely linked to MCP–1 (elevated manifold in autism) and other proinflammatory chemokines, 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 regulated by calcium–dependent CREB.

In addition to several mutations in genes belonging to major histocompatibility complex (MHC II) region, related to immune function and associated with autism, the involvement of CREB–mediated events in regulation and expression of these genes should be of utmost interest [16730065, 10458754] (see Brain_Development).

Some aspects of calcium signalling in immunity and inflammation

Proinflammatory cytokines/chemokines are protein signals released from a variety of cells in response to bacterial or viral infections or agents that cause physical damage, for example oxalates, in order to attract leukocytes to the site of inflammation. CC chemokines induce the migration of monocytes and other cell types such as NK cells and dendritic cells. One such
example is MCP–1, which induces monocytes to leave the bloodstream and enter the surrounding tissue, becoming tissue macrophages.

Many inflammatory processes are mediated through activities of LTCC and elevation in intracellular calcium level is the central element in the activation of brain macrophages. Several calcium channel blockers at therapeutic concentrations were shown to modulate inflammatory processes. Benidipine for example suppressed induction of MCP–1 and IL–8 [15364009]. In another study nifedipine inhibited TNF–alpha–induced reactive oxygen species generation and subsequent MCP–1 [15921025, 15018305]. The activity of LTCC mediates the cyclic stretch–induced inflammatory gene expression in airway smooth muscle and can be inhibited by nifedipine, with the most responsive genes being the ones encoding for COX–2, IL–6, and IL–8 [16339998, 8576944]. The induction of lymphocyte migration by IL–8, IL–1 alpha and IL–1 beta also appears to be dependent on activities of VGCC and can be inhibited by several calcium channel blockers [2686646]. TNF–alpha concentrations are shown to be suppressed in animals pretreated with verapamil or diltiazem, and by nimodipine in cultured monocytes [15661163]. On the other hand calcium entry blockers increase the regulatory interleukin–10 production, shown to be consistently low in autism [9110418].

Of special interest should be several recent observation of reduced responsiveness to stimulation of both B and T lymphocytes following elevations of cytosolic free calcium [12805281] (see Infectious Agents). Although LTCC are mainly expressed in excitable cells, recent evidence points to the involvement of voltage–insensitive dihydropyridine channels in calcium pathways of both B and T lymphocytes [8631746, 9550376, 14981074]. Calcium influx through the plasma membrane of lymphocytes is essential for their activation, proliferation, cytokine secretion and apoptosis [16766050]. Calcium and CREB–mediated upregulation of chemokine receptors mRNA has been observed in T lymphocytes, whereby activation of CXCR4 by viral proteins leads to increased viral infectivity [11874984, 14563373].

Furthermore, differences in calcium mobilization are associated with differentiation of naive CD4+ T cells into Th1 and Th2 subsets. Because LTCC are induced during Th2 but not Th1 cell differentiation, it has been suggested that LTCC blockers may be useful in the treatments of Th2–mediated pathologies. One such agent, nicardipine, was able to inhibit the Th2–mediated autoimmune glomerulonephritis induced by injecting Brown Norway (BN) rats with heavy metals, but had no effect on Th1–mediated experimental encephalomyelitis [15100258, 15777162, 14708347, 12721099].

Apoptosis plays an important role in the control of the immune system, and its impairment may be associated with autoimmune responses. Calcium entry through LTCC is thought to play a central role in apoptosis–related processes, including apoptotic body engulfment by dendritic cells.

Alterations in intracellular calcium levels have been implicated in the pathophysiology of immune dysfunction. Elevation of calcium levels play a central role in the regulation of executive functions in activated microglia, the immunocompetent cells in the brain, which have many similarities with macrophages of peripheral tissues and also express a variety of ion channels. Activation of microglia has been observed during infection, inflammation, physical injury, trauma, ischemia, and in a number of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, and prion disease [12805281, 9914452]. It has been hypothesised that calcium influx via VGCC plays a significant role in the development of neurological disability and white matter damage in the animal experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), and MS itself. Administration of calcium channel blockers significantly ameliorated EAE in mice, with spinal cord samples showing reduced inflammation and axonal pathology [15296830]. It should also be mentioned in this context that phosphodiesterase (PDEIs) inhibitors have shown inhibiting effect on cytokine production in lipopolysaccharide–stimulated mouse microglia. PDEIs supressed the production of TNF–alpha, as well as IL–1 and IL–6. In contrast, the production of IL–10, was upregulated by certain PDEIs, and it was concluded that these agent may be useful in treating inflammation in the CNS [10335522]. One of the possible explanations for the anti–inflammatory effect of these agents is their regulatory effect on LTCC [7517800].

Mast cells are important for clearance of pathogens and in wound healing, and play a central role in many forms of allergies, like asthma and eczema. Upon stimulation mast cells degranulate,
releasing histamine, prostaglandin and many cytokines. Because of their ability to release a wide range of cytokines and other inflammatory mediators, they are thought to have a central role in innate immunity and are implicated in the pathology associated with inflammatory and autoimmune disorders such as rheumatoid arthritis and multiple sclerosis. The activation and release of both plasma cells and mast cell contents is regulated by the influx of calcium, most probably through Cav1.4 LTCC, which are highly expressed in those cells [9357819].

Relative to autism it may be of interest to mention that a specific mechanism has recently been suggested, whereas the crosstalk between chemokine receptors and neuropeptide membrane receptors serves as a bridge between the immune and nervous systems. Chemokine receptors, a family of G protein–coupled receptors, are widely expressed by cells of immune and nervous systems, including neurons. The activation of several other receptors, such as opioid, vasoactive intestinal peptide, or adenosine receptors, often has inhibitory effects on chemokine receptors by a mechanism termed heterologous desensitization, resulting in suppression of immune responses. Conversely, activation of chemokine receptors also induces desensitization of mu-opioid receptors (see Infectious_Agents and Related_Receptors). In addition to that, prior exposure of neuronal cells to chemokine treatment enhances the sensitivity of transient receptor potential vanilloid 1 (TRPV1) calcium channel, which is critical for sensing of pain [16204635] (see Sensory/Motor).

Apart from playing an important role in functioning of lymphocytes, chemokines can potentially influence neuronal signaling through the modulation of neuronal calcium currents (see chapter on Chemokine Receptors and Calcium Homeostasis in the Brain). Following interaction with their specific ligands, chemokine receptors trigger a flux in intracellular calcium ions in neurons. This process is sensitive to treatment with pertussis toxin, indicating the involvement of G–protein coupled receptors in these mechanisms (see above) [11880151].

In the CNS, activation of neuronal chemokine receptors by their ligands induces calcium transients and activates calcium cAMP–dependent gene transcription factor CREB [9826729].

Similarly, some chemokines are able to induce a rapid and transient rise in cytosolic free calcium in in either type of T–cell via activation of their ligands [7926371]. It may be of interest to mention here a novel model of reversible inflammatory encephalopathy that is found to be dependent on both genetic and environmental factors. In a study that investigated the pertussis toxin–induced encephalopathy in mice, it was shown that transgenic mice that overexpress MCP–1 manifest transient, severe encephalopathy with high mortality after injections of pertussis toxin, whereas this disorder was significantly milder in mice lacking T–cells. Disruption of CC chemokine receptor 2 (CCR2) abolished both CNS inflammation and encephalopathy in this case [12486156] (see Bacterial lipopolysaccharides).

Several viral proteins that are able to directly interfere with human chemokine receptors have been identified. In the case of the most widely–studied HIV–1, two of its proteins mimick chemokine–mediated calcium signaling and evoke calcium influx through LTCC via activation of several chemokine receptors. One of the chemokine receptors involved, CXCR4, is known for its involvement in immune dysfunction thought to be due to dysregulation of the receptor leading to enhanced calcium signaling [17169327] (see Infectious_Agents). Through this mechanism it is thought to be able to modulate the migratory and secretory responses of microglia or cause raises in calcium levels in neurons resulting in neuronal injury [10858625, 16553776].

Human herpesvirus 6 (HHV–6) encodes highly effective and adaptive chemokine receptor agonists, which will attract CCR2 expressing cells, including macrophages and monocytes. At the same time these proteins enable viral interference in calcium signalling pathways and thus influence many downstream cellular processes. HHV–6 encoded U83A gene is able to induce significant calcium mobilization in cells expressing chemokine receptors CCR1, CCR4, CCR6, or CCR8, with high affinity to both CCR1 and CCR5 on monocyctic/macrophage cells. It was concluded that this newly uncovered mechanism could have wider implications for neuroinflammatory diseases such as multiple sclerosis, where both cells bearing CCR1/CCR5 plus their ligands, as well as HHV–6A, have been suggested to play etiological role [16332987, 16365449]. (also see BBB)

Virus–induced immune suppression has been documented with numerous viruses including other members of the herpes virus family, which are able to lower antigen presenting ability of myocytes via impaired differentiation of monocytes to dendritic cells, impaired migration of dendritic cells
[15914842], loss of up regulation of MHC protein (both Class I and Class II) expression, and loss of up regulation of CD4 cells and CD8 cytotoxic cells, altered cytokine production and activity, altered T cell recognition, and loss of NK function with lack of viral killing [16647570, 1315587, 9087413]. CD4 and CD8 cytotoxic cells and NK cells are affected either directly or indirectly by HHV-6 [9227865, 1348547]. The impairment of immune function by herpes and other viruses may lead to reactivation or emergence of other opportunistic micro-organisms that persist chronically or in a latent state, for example Epstein Bar Virus (EBV), chlamydia, mycoplasma, Borrelia burgdorferi, and babesia, among others. Worth mentioning in this context is the finding that a large subset of subjects with autism shows evidence of various bacterial and/or viral infections not present in age-matched controls (see Infectious_Agents).

It is suggested that one of the mechanisms through which herpesviruses exerts its immunosuppressive effects is via dysregulation of calcium homeostasis in immune cells [14568989]. Another possible example of this mechanism is the effect of HIV–1 viral protein Tat, which is known to inhibit functioning of dendritic cells and NK cells by dysregulating the functioning of LTCC [9516412, 9743356]. In cultured human primary monocytes Tat induces a calcium signal by mobilizing calcium from extracellular stores. This effect is again mediated via calcium influx through LTCC [15661163]. Another viral protein, belonging to Hepatic C, is known to alter expression of genes involved in cell adhesion and motility, calcium homeostasis, lipid transport and metabolism, as well as genes regulating immune and inflammatory responses [15349911].

Apart from viruses various other agents are thought to have immunosuppressing properties and can act as stressors, negatively influencing proliferative responses of cells. Various toxic chemicals have been shown to induce immune dysregulation via their effects on calcium homeostasis. Cadmium-induced increases in intercellular calcium levels in macrophage cells lead to cellular dysfunction and cell injury, including growth arrest, mitochondrial activity impairment, and necrosis [15254339]. Exposure of cultured murine macrophages to low concentrations of beryllium also induced increases in intracellular calcium and subsequent DNA synthesis. This effect could be reversed by removing extracellular calcium or applying verapamil, indicating the involvement of plasma membrane calcium channels [10380900]. Tin appears to exert similar effects on immune cells via calcium mobilisation [9138773].

Mercurial compounds are particularly toxic to the human immune system. Chronic low-level methylmercury (MeHg) exposure may cause immune disfunction by increasing oxidative stress, affecting transmembrane calcium signaling and splenic cellularity [9653674]. In cultured murine T and B lymphocytes mercury induces cytotoxicity via several mechanism that involve disturbances in calcium homeostasis and inflammatory cytokine gene expression [12849721]. Adverse affect of methylmercury on the developing immune system through placental and lactational transfer has been demonstrated in rats [1949074]. Intracellular levels of free calcium play an essential role in neutrophil activation, and methylmercury increases those levels in the mouse peritoneal neutrophils via LTCC [12062935]. Exposure of human cultured lymphocytes to methyl mercuric chloride (MeHgCl) results in a sharp decrease in the mitochondrial transmembrane potential in lymphocytes, followed by an increase in oxidative stress [12215206]. Dendritic cells, especially immature dendritic cells, appear to be very sensitive to the effects of organic compound ethylmercurithiosalicylate (thimerosal, primarily present in the tissues as ethylmercury) on calcium homeostasis. Uncoupling of ATP-mediated calcium signaling and dysregulated interleukin–6 secretion has been observed in murine cells following application of relatively small levels thimerosal, with one mechanism involving the uncoupling of ryanodine receptor RyR1 calcium channel complex [16835063]. It should be noted here that in many cells types ryanodine receptors of endoplasmic/sarcoplasmic reticulum form a functionally and structurally connected signalling unit with LTCC on the plasma membrane, and this signaling interaction between LTCC and RyRs is often bi-directional, in that the LTCC trigger the release of intracellular calcium by promoting the opening of nearby RyR (orthograde coupling) while at the same time the activities and function of LTCC can regulated by its interaction with the RyR (retrograde coupling) [11861208].

(see also chapter: Genetic Polymorphisms as Predictors of Inflammatory Outcomes and Chemokine Receptors as Treatment Targets)
Gastrointestinal issues

Gastrointestinal findings 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 behavior 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 pancreatico-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. Compared with healthy subjects, serum levels of 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, parallel to the above-mentioned findings in autism (17720995).

Some aspects of calcium signalling in GI tract

Gastric acid is one of the main secretions of the stomach – if its production is insufficient the risk of gastrointestinal infections and of developing gastroenteritis is greatly increased, while on the other hand excessive production, which can sometimes be caused by hypercalcemia, can cause gastric ulcers. Voltage gated calcium channels are thought to be involved in the regulation of gastric acid secretion. Calcium channel blocker nifedipine was shown to significantly reduce gastric acid secretion, and verapamil, diltiazem, cinnarizine, nifedipine and hydralazine showed similar effects (7721557, 4072828). Calcium influx through the plasma membrane as controlled by VGCC is also involved in fluid secretion by the enteric nervous system [9338518].

Gut motility, especially during inflammation, also seems to be mediated by calcium flux [10973625] [11498505]. In one study verapamil, a calcium channel blocker, was able to inhibit giant migrating contractions and diarrhea during small intestinal inflammation [9357819]. Abnormalities in muscle contraction and digestion in inflammatory bowel disease, such as ulcerative colitis or Crohn's disease, are associated with disturbances of calcium homeostasis in the inflamed muscle cells [10492128, 11159888]. Inflammation of the colon results in changes in ion channel activity of smooth muscle cells [10964716].

Another aspect of the inflammation of the intestine is that it may be a subject to oxidative stress. Treatment of isolated segments from the rabbit jejunum and from the guinea pig ileum with free radicals resulted in dysfunctions of contractility. This effect was reversed by Bay-K 8644, which activates LTCC, suggesting that oxidative stress might have a direct effect on calcium entry through these channels [12457627] (see Oxidative Stress).

It has been suggested that oxygen free radicals and calcium influx may play a role in the development of endothelial barrier injury, possibly leading to intestinal hyperpermeability [8578176]. Under certain conditions intestinal epithelial cells may become a source of proinflammatory cytokines, which actively contribute to ongoing inflammation through autocrine disruption of epithelial barrier function 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, an event regulated mainly through influx of calcium via VGCC [15253703] (see also Hypoxia and Brain).

In addition to endothelial cells, calcium homeostasis plays a central role in the maintenance tight junctions, which represent the major barrier between intestinal cells and whose disruption can also lead or contribute to intestinal hyperpermeability, or "leaky gut" (see BBB).

Calcium flux through plasma membrane plays a crucial role in secretory functions of pancreas. It is the main mechanism for release of insulin and regulation of insulin synthesis – significant increases of calcium levels in the cells causes release of previously synthesised insulin, which has previously been stored in secretory vesicles. The calcium level also regulates expression of the insulin gene via the calcium responsive element binding protein (CREB), which is central in beta-cell gene expression and function [16908541]. In addition, pancreatic fluid and enzyme secretion is dependent on extracellular calcium and its entry through plasma membrane [6257554, 6121340]. Inappropriate regulation of beta-cell CaV channels causes beta-cell dysfunction. Glucose-stimulated insulin secretion depends on calcium influx through voltage gated channels [171488757] and a mutation in the human CaV1.2 gene results in excessive insulin secretion. Trinucleotide expansion in the human CaV1.3 and CaV2.1 gene is revealed in a subgroup of patients with type 2 diabetes [16868246]. It is of interest to note the role of glutathione: in pancreatic islets insulin secretion in response to a variety of stimulators is sensitive to the redox state of extracellular and intracellular thiols. One major localization of critical thiols appears to be related to the influx of calcium through VGCC [2424631].
**Dysregulating factors**

Several infectious agents have been shown to have direct dysregulatory effects on calcium homeostasis in the gastrointestinal tract. HIV-1, one of the most widely studied viruses, may directly alter ion secretion in the intestine, which is suggested to be the mechanism behind the watery diarrhea associated with HIV-1 infection [7583886]. The HIV-1 transactivating factor protein (Tat) induces ion secretion in Caco-2 cells and in human colonic mucosa similar to that induced by bacterial enterotoxins. It also significantly prevents enterocyte proliferation. Increase in intracellular calcium concentration and the antiproliferative effects of Tat are mediated by L-type calcium channels [12557143].

In pancreatic acinar cells calcium overload is an early event in the pathogenesis of cell damage and dysregulation of calcium homeostasis is be one of the main causes or mediators of pancreatic acinar cell damage induced by bacterial lipopolysaccharides [12904279, 10795755]. Calcium-blocking agents, such as magnesium and tetrandrine, have shown protective effects against damage to acinar cells induced by such calcium overload [16524508, 11259384].

Rotavirus infection induces increases in intracellular calcium concentration in human intestinal epithelial cells. Rotavirus protein NSP4 induces increased membrane permeability, calcium influx and diarrhea when administered to mice, an effect thought to be age-dependent and mediated through calcium dependent chloride secretion and calcium-induced impairment of nutrient digestion [12438636, 11044126, 7637021].

Inflammatory effects of Clostridium Difficile toxins in the intestine may be related to their ability to induce elevation of cell calcium levels, both by mobilising it from intracellular stores and by provoking calcium influx from the extracellular space [11413111, 2838520, 7900810].

Acute and chronic gastrointestinal diseases are known to be commonly caused by viral infection of GI tract. The symptoms are though to be the result of damage to the villi, but there are also indications of active secretion and motility disturbances. Rotaviruses are known to cause acute gastroenteritis – the virus infects enterocytes of the villi of the small intestine, leading to structural changes of the epithelium and diarrhea, sometimes followed by vomiting and low-grade fever. Rotaviruses tend to affect gastrointestinal epithelial cells that are at the tip of the villus. Their triple protein coats make them very resistant to the normally prohibitive pH of the stomach, and also to digestive enzymes (lipases and proteases) in the GI tract. Temporary lactose intolerance has also been associated with rotavirus–induced gastroenteritis. [17031143, 2803034]. Rotavirus was also shown in one study to affect amino acid uptake in the small intestine. Infected mice showed significant reduction in their bodyweights and intestinal lengths compared with controls. Gluthatione uptake was amongst others to be significantly reduced. Findings showed damage to the villi in the jejunum and prominent cytoplasmic vacuolation in the ileum of infected animals [10482428]. Rotavirus pathogenesis and gastrointestinal symptomatology is though to be age-dependent. In addition, increasing line of evidence suggests that rotavirus is able to spread to a number of different organs in the body, including the brain, where it can cause neurological disturbances, most notably seizures and loss of language and social interaction (see Infectious_Agents).

Viruses can remain latent in gastrointestinal tissues and produce disease many years after initial infection. Latent herpes simplex virus type 1 gene expression is prevalent in human adult nodose ganglia, suggesting that infection of gastrointestinal sensory nerves probably occurs commonly and that HSV–1 reactivation from this site may play a role in recurrent gastrointestinal disorders [9094690]. In one study a reactivation of latent herpesviruses was identified in several children with active inflammatory bowel disease [2298361]. Murine gammaherpesvirus–68, used to study human EBV and HSV–8 viruses, was shown to induce a systemic lymphocytosis in mice and to establish a latent infection of lymphocytes [10644841]. Two herpesviruses, cytomegalovirus and herpes simplex virus, can cause ulcerative disease of the gastrointestinal tract.

Acute gastroenteritis occurs in healthy persons but it is thought to be more common and more severe in immunocompromised patients. In patients with Acquired Immunodeficiency Syndrome (AIDS) and immunocompromised hosts, CMV can cause primary, latent or chronic persistent infection. Primary CMV infection is very severe in immunocompromised patients as well as among healthy population. Among patients with AIDS CMV is usually isolated from patients specimen in association with other pathogens (Pneumocystis carinii, Candida albicans). There is high
prevalence in AIDS population of serious CMV-related diseases, including chorioretinitis, gastrointestinal disease, interstitial pneumonia and central nervous system disease [10872268] (see also Immune/Inflammation).

Epstein-Barr virus, human papilloma virus, and human herpesvirus-8 are implicated in proliferative diseases of the gastrointestinal tract. Epstein–Barr virus has been associated with immunoproliferative disease after transplantation and may also cause small-bowel and colonic lymphoma. Human papillomaviruses cause anorectal condyloma and anal cancer. Human herpesvirus-8 causes gastrointestinal Kaposi sarcoma [10980963]. Intestinal CMV-infected cells in infants have prevalently been associated with neonatal necrotizing enterocolitis. A case report of an infant with congenital or perinatal CMV infection with gastrointestinal involvement describes inflammation in the GI tract, development of a colonic stricture and manifested a clinical picture simulating Hirschsprung’s disease (characterised by bowel obstructions, megacolon, protruding abdomen). Chorioretinitis was also present [16567208].

It has been hypothesized that both chronic gastritis and ulcerative colitis are induced by viral infection, and that such chronic infection of the mucosa may lead to ulceration and occasionally cancer [7321919].

A case report on a 12 week old fetus that was aborted following herpes family varicella-zoster virus (VZV) infection in mother, viral infection was identified in fetal dorsal root ganglia, meninges, gastrointestinal tract, pancreas, smooth muscle, liver, and placental trophoblast inclusions (see Infectious_Agents and Maternal_Factors), indicating the presence of a nonproductive, latency-like VZV infection. It was concluded that widespread nonproductive infection in the absence of histological clues is an early event in VZV infection in fetuses. Latency-like infection in nonneural cell types may potentially reactivate, leading to multifocal necrosis, fibrosis, and dystrophic calcifications, as observed in advanced congenital varicella syndrome [15655777].

In recent years there have been increasing evidence of the involvement of measles virus in ileocolonic lymphonodular hyperplasia – a new form of inflammatory bowel disease that has been described in a cohort of children with developmental disorders. Seventy five of 91 patients with a histologically confirmed diagnosis of ileal lymphonodular hyperplasia and enterocolitis were positive for measles virus in their intestinal tissue compared with five of 70 control patients. Measles virus was identified within the follicular dendritic cells and some lymphocytes in foci of reactive follicular hyperplasia [11950955].

In the light of recent discoveries of critical roles played by several chemokines and their receptors in lymphoid development, mucosal immunity, and intestinal inflammation, possible upregulation of these receptors in autism and downstream consequences on calcium signalling would merit closer investigation. Based on the fact that chemokines and their receptors are crucial mediators of inflammation and tissue injury, it is now believed that their antagonists could provide novel therapeutic tools in the treatment of inflammatory bowel disease [11149563, 11872088]. Several genetic polymorphisms related to chemokine receptor expression play a role in vulnerability of gastrointestinal tract to infectious agents. A good example is that genetic deficiency in the chemokine receptor CCR1 was shown to protects against acute Clostridium difficile toxin A enteritis in mice – while the toxin induced in all mice a significant increase in ileal fluid accumulation, epithelial damage, and neutrophil infiltration, all parameters were significantly lower in CCR1 and MIP–1alpha knockout mice [11875005].

(see Infectious_Agents)

In addition to infectious agents, various toxins are known to affect functioning of GI tract [9181600, 8699562] (see Toxins).
**Autism, membrane metabolism and calcium homeostasis**

Smith–Lemli–Opitz Syndrome (SLOS) is a rare genetic condition of impaired cholesterol biosynthesis, with most of affected individuals simultaneously exhibiting at least some of the symptoms of autism. The figures on the rates of formal autism diagnosis in individuals with SLOS vary but appear to be somewhere between 50–75 percent, among the highest of single gene disorders associated with autism [16761297]. While supplementing dietary cholesterol frequently eliminates or ameliorates many of the feeding and growth problems of SLOS, it has been observed recently the autistic behaviors of children with SLOS can also be reduced or even eliminated by treatment with supplementary dietary cholesterol. In addition to behavioural and language–delay problems, individuals with SLOS often suffer from several gastrointestinal problems, including severe reflux and constipation, immune deficiency and sleep problems.

Disturbances in membrane sterol content in SLOS is thought to directly contribute to various cellular abnormalities in observed in this disease, which include significantly increased calcium permeability as well as reduced folate uptake (see Oxidative Stress). Most interestingly, partial restoration of the excessive calcium influx pathway was observed following cholesterol enrichment [16258167]. Increasing evidence indicates that membrane cholesterol is capable of modulating function of voltage gated calcium channels. Caveolae, including caveolae–like plasma domains in neurons, and transverse (T–) tubules are membrane lipid–raft structures rich in caveolin, cholesterol and glycosphingolipids, as well as VGCC. Recent results have shown that a depletion of membrane cholesterol alters caveolae and T–tubules. Cholesterol and LTCC occupy a similar molecular location in the membrane. In one study application of a a cholesterol–sequestering drug resulted in significant reduction in caveolae and T–tubule areas and to a significant reduction of LTCC current, suggesting that membrane cholesterol content modulates their function, and that both lowered and excessive cholesterol levels can modulate calcium currents [14724204, 2054935]. This is proposed to be one possible mechanism behind the observed reduction in autistic symptoms in SLOS following supplementation of dietary cholesterol. Another possible mechanism to be taken into consideration is the effect of cholesterol on several serotonin receptors and G–protein and ligand bindings of those receptors (see Neurotransmitters).

In another study the addition of Caveolin–1 to cultured neurons resulted in increased levels of membrane cholesterol and reduction in calcium currents, thus showing that caveolin–1 also influences neuronal VGCC activity [16040758].

Following the above findings, it is proposed that the opposite effect might exist, whereas dysregulation of VGCC could under certain conditions directly influence the membrane structure, including the synthesis and/or levels of cholesterol and caveolin [11353331]. In support of this hypothesis is the finding from human fertility studies demonstrating that cholesterol synthesis can be significantly increased in sperm treated with nifedipine, a calcium channel antagonist [link].

Abnormalities of cholesterol metabolism have been observed in more common forms of autism, with substantial numbers of individuals showing lowered levels as compared to controls [16874769], while elevated levels of cholesterol have been observed in individuals with Asperger's syndrome [17123635]. In addition to cholesterol levels, results of a study investigating brain high energy phosphate and membrane phospholipid metabolism provided further evidence of undersynthesis and increased degradation of brain membranes in autism. It was observed that membrane building blocks decreased, and levels of membrane breakdown products increased parallel to a decline test performance abilities in subjects with autism [8373914].

In addition to the above, it is worth mentioning that one of the downstream reactions in neurons following elevation in the levels of intercellular calcium is the activation of several lipases, proteases, and endonucleases that attack the structural integrity of the cell. Calcium also activates phospholipase C, which promotes a progressive breakdown in the phospholipid components of the both plasma and intercellular membranes.
Oxidative stress

Oxidative stress in autism – overview of genetic and environmental factors

Oxidative stress is defined as an imbalance between pro-oxidants and anti-oxidants, resulting in damage to cell by reactive oxygen species (ROS). Reactive oxygen species include oxygen ions, free radicals and peroxides. They form as a natural byproduct of the normal metabolism of oxygen and have important roles in a number of biological processes, such as the killing of bacteria. 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. Vitamin E plays an important role in cellular defence against lipid peroxidation – a degradation of cell membrane by free radicals.

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). The authors propose that an increased vulnerability to oxidative stress may be a contributive factor to the development and clinical manifestations of autism [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).

Several murine studies showed that defective homocysteine remethylation can be caused by deficiency of either methionine synthase enzyme that catalyzes the folate–dependent remethylation of homocysteine to methionine or deficiency of folic acid that produces oxidative stress and endothelial dysfunction in the cerebral microcirculation. [16043641].

In addition to genetic defects such as cystathionine beta–synthase (CBS) or MTHFR, additional factor that can contribute to increasing plasma homocysteine levels is the nutritional status of vitamin B12, vitamin B6, or folate deficiencies.

Folate deficiency induces neurotoxicity by multiple routes – apart from increasing oxidative stress by increasing the levels of homocysteine, it can also contribute to increases in cytosolic calcium and to subsequent mitochondrial and DNA damage (see Mitochondria). Folate deprivation was shown to induce calcium influx initially through the LTCC, and subsequently through NMDA channels and from internal stores [15038821].

Oxidative stress and calcium signalling

Recent data supports the speculation that calcium and reactive oxygen species are two cross-talking messengers in various cellular processes. The results of various studies have shown that calcium is essential for production of ROS. Elevation of intracellular calcium level is responsible for activation of ROS–generating enzymes and formation of free radicals by the mitochondria respiratory chain. It has been shown that the effective macrophage redox defense against Chlamydia pneumoniae depends on LTCC channel activation [12736823]. Hydrogen peroxide, a membrane–permeable form of reactive oxygen species, was shown to enhance inward calcium current in cultured dentate granule cells. This enhancement was cancelled by glutathione, an antioxidant, and nifedipine, an LTCC channel blocker, suggesting that oxidative stress induced by hydrogen peroxide selectively regulates the activity of LTCC [14746893, 9152045]. Nimodipine, another calcium channel blocker, was also shown to suppress ROS formation [10601165, 15820440, 9489715], and verapamil was both protective against oxidative stress and ameliorated morphological changes and dysfunction of mitochondria (see Mitochondria) [16644187].

On the other hand, an increase in intracellular calcium concentration may be stimulated by ROS. Hydrogen peroxide has been recently shown to accelerate the overall channel opening process in voltage–dependent calcium channels in plant and animal cells. In addition to outer membrane calcium channels, IP3 receptors as well as the ryanodine receptors of sarcoplasmic reticulum have also been demonstrated to be redox–regulated [14616077].

The rise in intracellular calcium activates, amongst other things, nitric oxide synthetases, a group of enzymes responsible for the synthesis of nitric oxide. A study looking into mechanisms of NO synthase in the developing rat cortex found that, quote: “... depolarization following GABA–A receptor activation leads to opening of L-type voltage–gated calcium channels, resulting in an increased calcium influx, which in turn leads to phosphorylation and, thus, activation, of the transcription factor CREB: the phosphorylated CREB can then induce BDNF, as well as nNOS ” [14604759, 9153595] (see also Brain_Development).

In another study, application of dihydropyridine calcium channel blockers had protective effects against endothelial cell oxidative injury due to combined nitric oxide and superoxide. Nisoldipine, nicardipine and nifedipine all attenuated oxidative–insult induced by loss of reduced glutathione, with nisoldipine demonstrating greatest protection [11820858].

It has been shown that in red blood cells increases in intracellular calcium concentrations lead to a decrease of membrane protein methyl esterification and a subsequent impairment of S–adenosylmethionine synthesis (SAM). After the removal of extra calcium from the cells the levels of methyl esterification returned to normal [3081340].

Excessive lipid peroxidation is implicated in the pathogenesis of neurodegenerative disorders and is brought upon by free radicals action on cell membrane in the absence of inadequate antioxidant defence. Lipid peroxidation has been shown to modulate the activity of VGCC. In one study a
prolonged exposure to a lipid-peroxidation enhancer resulted in neuronal death, which was prevented by treatment with glutathione and attenuated by the LTCC blocker nimodipine. It was concluded that the modulation of calcium channel activity in response to lipid peroxidation may play important roles in the responses of neurons to oxidative stress in both physiological and pathological settings [12006588].

Homocysteine is found to overstimulate NMDA receptors, leading to excessive calcium influx and possible neuronal damage [10797837].

Similar to the abovementioned cross talk between ROS and calcium, glutathione, as well as being influenced by calcium, also seems to have a critical role in gating the VGCC. Inhibition of glutathione reductase by carmustine in vitro resulted in depletion of glutathione and oxidative stress, and an influx of extracellular calcium through LTCC. This increase in intracellular calcium was dependent on the presence of extracellular calcium and could be inhibited by calcium blockers nimodipine or nitrendipine. In addition, this effect was also suppressed in cells that were treated with an antioxidant deferoxamine, and enhanced in cells that were pretreated with an inhibitor of glutathione synthesis, buthionine sulfoximine [15321730].

It may be of relevance to note that calcium channels in pancreatic islets are very sensitive to levels of glutathione. Membrane thiols are thought to play an important role in insulin secretion due to their effect on calcium influx via those channels [2424631]. (see Pancreatic function/GI)

Causes of oxidative stress

Apart from poor nutritional status and/or genetic factors, various environmental agents have also been implicated as causative agents in disturbances in methylation pathways and increased oxidative stress. Oxidative damage due to increased generation of reactive oxygen species and reactive nitrogen species is a feature of many viral infections. The increasing prevalence of HIV-associated cognitive impairment has been the subject of many recent studies, the result of which provide overwhelming evidence for oxidative stress in mediating neuronal injury in patients with HIV induced dementia. These studies also suggest that patients with apolipoprotein E4 allele are more susceptible to neuronal oxidative damage [17034352].

Raised homocysteine levels alongside folate deficiency has been observed in HIV infected children [11737242] and in other viral and mycoplasmal infections, including influenza A and B, human parvovirus, rubella, infectious mononucleosis and Mycoplasma pneumoniae [3033086] [12214730].

One study looking at absorption of folate in HIV infected patients has found that absorption of folic acid appears to be significantly impaired in HIV disease, irrespective of the stage of the disease or gastro-intestinal complaints. The authors presented data to support their hypothesis that the virus can cause an enteropathy in the absence of opportunistic infection [1680150].

Hepatitis C induced oxidative stress is also widely studied, with numerous studies showing Hepatitis infection causing a state of chronic oxidative stress. These viruses have been associated with changes in mitochondrial structure and function, including increased calcim uptake [16958669]. (see Mitochondria)

Furthermore there seems to be a direct correlation between levels Hepatitis A, B and C viral infections and levels of glutathione, whereby increased viral activity precedes decreased glutathione levels [11366543, 17036398].

Oxidative injury is also a component of acute encephalitis caused by herpes simplex virus type 1, reovirus, murine leukemia virus, and subacute sclerosing panencephalitis caused by measles virus [15944946].

Various environmental toxins have been shown to cause oxidative damage to the cell. Metal-mediated formation of free radicals causes various modifications to DNA bases, enhanced lipid peroxidation, and altered calcium and sulfhydryl homeostasis. For a group of metals that include mercury, cadmium and nickel, one route for their toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins [15892631] [8512585].
A study looking at the effects of both methylmercury and inorganic mercury on cell oxidative stress and intracellular calcium concentration in rat cerebellar granule neuron cultures tested neuroprotective effects of several agents that selectively interfere with these mechanisms. The results suggested that disruption of redox equilibrium and calcium homeostasis contribute equally to inorganic mercury cell damage, whereas oxidative stress is the main cause of methylmercury neurotoxicity [11599010].

A study looking at functional activities of insulin-like growth factor-1, dopamine-stimulated methionine synthase, and folate-dependent methylation of phospholipids in normal development found that these pathways were interrupted by neurodevelopmental toxins, such as ethanol and heavy metals. Study results raised the possibility that these toxins might exert adverse effects on methylation pathways [14745455]. Reversible alterations of oxidative stress biomarkers resulting from in utero and neonatal exposures of airborne manganese have also been documented [16943606]. (see also Toxic_Agents)

Investigation of cellular mechanisms affected by lowered membrane cholesterol in Smith-Lemli-Opitz syndrome found decreased folate uptake parallel to enhanced calcium permeability (see Membrane).

Additional issues for consideration: implications of calcium signalling in oxalate formation and deficiencies of magnesium and B vitamins

Deficiencies of several forms of vitamins B have been shown in vitro and in rodent studies to enhance calcium influx through VGCC. For example it has been observed that depolarization and activation of LTCC occur during experimental thiamine deficiency, and it was proposed that this mechanism may play a role in histological brain lesions/damage induced by thiamine deficiency [9669323].

In the context of reports on beneficial effects of supplementation of vitamin B12 in autism, as well as simultaneous supplementation of magnesium and vitamin B6 [links], it is of interest that several studies observed that simultaneous deficiencies of magnesium and pyridoxine may in fact act synergistically on impairing the function of LTCC. Deficient rats were found to exhibit excessive influx of calcium into the intracellular compartments. Lowering magnesium in resulted in elevation of calcium in cultured canine cerebral vascular cells, and this calcium entry could be blocked by exposing the cells to vitamins B6, B12, or folic acid, simultaneously or individually [10553943, 1645979, 9823019].

Of some relevance could be the observation that simultaneous application of magnesium and pyridoxine significantly decreased formation of oxalate in a small-scale human study [7992461]. It is proposed that regulation of calcium fluxes through LTCC by these agents may at least in part underlie their effects in mammals, as it does in plants [link], as application of various calcium channel blockers has been shown to decrease oxalate formation in human and in animal studies. [8322624, 1845698, 10354288]

Conclusion:

Following the above findings it is suggested that folate and cobalamin deficiencies observed in neurological disorders may be caused by a combination of genetic polymorphisms as well as impaired absorption and disturbances in metabolic pathways due to viral and other infections and environmental toxic load, mediated in great part through enhanced calcium signalling. The involvement of viral infections and environmental toxins as causes of disturbed folate metabolism and oxidative stress and their relation to calcium homeostasis may be of relevance to autism and neurological disorders and requires further investigation.
Mitochondrial dysfunction

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). Disturbance of mitochondrial energy production in autism was confirmed by another recent study [19043581]. (also see Brain Development and Oxydative Stress).

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] (also see Membrane chapter). 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 Oxidative Stress chapter)

Calcium homeostasis and mitochondria

One of the functions of mitochondria is to store free calcium. Release of this stored calcium back into the interior of the cell can initiate calcium spikes or waves. These events coordinate various processes in different types of cells, for example neurotransmitter release in nerve cells and release of hormones in endocrine cells. Excess calcium ions stored in mitochondria can inhibit oxidative phosphorylation. In the nerve cells this can causes an irreversible reduction in the energy status of nerve terminals, which can initiate pathophysiological processes in those cells.

Numerous findings have indicated a crucial role of calcium influx through L-type calcium channels in mitochondrial calcium overload and downstream mitochondrial and cellular dysfuctions. It has been shown that blockade of LTCC in the plasma membrane not only inhibits an increase in cellular calcium but also stabilizes mitochondrial membranes calcium homeostasis and generation of ROS by mitochondria [16760264, 11746731]. In one study inhibition of calcium inward current with verapamil protected against oxidative stress as well as morphological changes and dysfunction of mitochondria [16644187] (Oxidative_Stress). There are some indications that, simultanious to LTCC, N-methyl-D-aspartate (NMDA) receptors are also involved in oxidative stress, mitochondrial dysfunction, and ATP depletion mediated by calcium influx [12473387].

The involvement of LTCC in cellular and mitochondrial accumulation of calcium has been demonstrated in vitro in hypoxic renal tubular cells [15339981], and in bovine chromaffin cells [11500491], showing that these channels play an important role in regulating mitochondrial permeability transition, cytochrome c release, caspase activation, and ATP depletion-induced mitochondrial apoptosis. The reduced efficiency of handling of intracellular calcium loads in neurons may be an important factor contributing to the onset of neuronal damage during hypoxia and ischaemia [8012725]. Calcium influx through LTCC is involved in the ischemic damage in neonatal brain which manifests itself as a decrease in the energy state, with decreased levels of phosphocreatine and ATP, and an increases in lactate [88974726] (see Hypoxia/Ischemia).

At the same time deenergization of mitochondria affects the cellular calcium influx rate [10930575]. Several inherited human encephalomyopathies exhibit neurological symptoms, including autism-related symptoms, in association with specific mitochondrial mutations [7846043]. It can therefore be proposed that this inability to regulate calcium influx and
homeostasis is one of the probable mechanisms behind increased neuronal vulnerability and subsequent development of autistic-like behavioural symptoms in human encephalomyopathies.

Male to female ratio in autism – the role of steroids and calcium signalling

Autism affects more boys than girls, the overall male to female in autism being 4:1. One possible explanation for this difference in prevalence is the role of androgens. For example, it has been suggested that prenatal exposure to high levels of testosterone influences some autistic traits and makes fetuses more susceptible to developing autism [16624315]. Testosterone is a steroid hormone from the androgen group. It is derived from cholesterol, with largest amounts being produced by the Leydig cells in the testes in men, and some being produced by the adrenal glands, ovaries and in the placenta. Estrogens function as the primary female sex hormone and are present at significantly higher levels in women. Estradiol 17beta is one of the major naturally occurring estrogens.

A growing number of studies in recent years have shed light on the mechanisms behind the effect of testosterone on gender-related differences in cardiac preformance. It has been observed that testosterone induces an increase in calcium by increasing expression and activity of LTCC in coronary arteries, in particular the expression of Cav1.2 [9166901, 16243844, 15114516, 15242831]. Testosterone-induced increases in calcium levels appear to be G-protein linked and sensitive to Pertussis toxin treatment, and involve emptying of intracellular calcium stores downstream from LTCC activation [16339199]. Testosterone potentiation of calcium channels has also been observed in several other cells [8969193, 1883394].

On the other hand an increasing line of evidence indicates that estrogen acts as both cardioprotective as well as neuroprotective agent, primarily by inhibiting LTCC and rises in intercellular calcium levels [17082253]. Estradiol is thought to influence various brain functions by acting on receptors on the neuronal membrane surface. Estradiol is thought to influence various brain functions by acting on receptors on the neuronal membrane surface. Many intracellular signaling pathways and modulatory proteins are affected by estradiol via this mechanism, including regulation of CREB, a stimulus–induced transcription factor that regulates various behaviors, including those related to addiction and chronic pain, as well as neuronal survival, proliferation, and differentiation (see Brain). One study has shown that estradiol attenuates CREB phosphorylation mediated by calcium influx through LTCC [15901789]. Administration of nifedipine, a calcium antagonist, mimicks the effects of estrogen on the peripheral nervous system [12732239]. Both nifedipine and estrogen protected neurons from amyloid–protein–induced toxicity through supression of calcium channel protein expression induced by this protein [15082219]. 17beta–estradiol was also able to attenuate glutamate–induced calcium overload in rat primary hippocampal neurons [15488487]. (see Hypoxia/Ischemia re protective effects of estrogen on brain arteries).

In addition, estrogen has been reported to have anti–oxidant properties – anti–oxidant effects of estrogen reduce intracellular calcium during metabolic inhibition and protect against damaging effect of calcium loading on mitochondria [12676548, 15723615] (see Mitochondria).

In terms of testosterone synthesis and secretion, calcium is an important modulator of Leydig cell steroidogenesis, which is mainly controlled by luteinizing hormone secreted from the anterior pituitary. Testosterone production in response to lutenising hormone is shown to be lower in the absence of extracellular calcium and in the presence of verapamil, a calcium channel blocking agent [9695359].

Testosterone formation in response to both lactate and to 4 beta–Phorbol–12–myristate–13–acetate (PMA) also appears to be dependent on extracellular calcium and could be blocked in vitro by the addition of the calcium channel blocking agents [2988910, 11500963].