

Microglia, Calcification and Neurodegenerative Diseases

Jose M. Vidal-Taboada, Nicole Mahy and Manuel J. Rodríguez
*Unitat de Bioquímica i Biologia Molecular, Facultat de Medicina, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED),
Barcelona
Spain*

1. Introduction

1.1 Neurodegeneration involve different cell types

Neurodegeneration is a complex process involving different cell types and neurotransmitters. A common characteristic of neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis, Huntington's disease (HD) and Amyotrophic Lateral Sclerosis (ALS) is the occurrence of a neuroinflammatory reaction in which cellular processes involving glial cells (mainly microglia and astrocytes) and T cells are activated in response to neuronal death. This inflammatory reaction has recently received attention as an unexpected potential target for the treatment of these diseases.

Microglial cells have a mesenchymal origin, invade the central nervous system (CNS) prenatally (Chan et al., 2007b) and are the resident macrophages in the CNS (Ransohoff & Perry, 2009). They comprise approximately 10-20% of adult glia and serve as the CNS innate immune system. In neurodegenerative diseases, microglia is activated by misfolded proteins. In the case of AD, amyloid- β ($A\beta$) peptides accumulate extracellularly and activate the microglia locally. In the case of PD, ALS and HD, the misfolded proteins accumulate intracellularly but are still associated with activation of the microglia (Perry et al., 2010). Reactive microglia in the substantia nigra and striatum of PD brains have been described, and increased levels of proinflammatory cytokines and inducible nitric oxide synthase have been detected in these brain regions, providing evidence of a local inflammatory reaction (Hirsch & Hunot, 2009). The injection of lipopolysaccharide (a potent microglia activator) into the substantia nigra produces microglial activation and the death of dopaminergic cells. These findings support the hypothesis that microglial activation and neuroinflammation contribute to PD pathogenesis (Herrera et al., 2000).

Astrocytes are ectodermal cells, and they are probably about ten times as numerous as neurons. Astroglial cells were initially believed to be passive support cells providing trophic support for surrounding neurons (Sofroniew & Vinters, 2010), maintaining extracellular ion homeostasis and capturing excess extracellular neurotransmitters such as glutamate, which is considered particularly important given its involvement in excitotoxicity. However, recent studies have implicated astrocytes in many complex CNS functions, such as physical

structuring of the brain (they are the main cells involved in cholesterol synthesis), active control of synaptogenesis and plasticity (Graeber, 2010), regulation of blood flow and promotion of myelination (Halliday & Stevens, 2011). Astroglial activation is characterized by an increase in expression of intermediate filament glial fibrillary acidic protein (GFAP) and the gene aldehyde dehydrogenase 1 family member L1 (ALDH1L1). Astrocytes are not immune cells per se, but they can, under specific conditions, contribute to the immune response (Farina et al., 2007).

Several other cell types have been associated with neurodegeneration and neuroinflammation, such as T-cells, oligodendrocytes and ependymal and subependymal cells (Philips & Robberecht, 2011). Once infiltrated in the CNS, T-cell subpopulations modulate the neuroinflammatory reaction differently, depending on stage of disease progression (Beers et al., 2011, Philips & Robberecht, 2011). Oligodendrocyte cells are widely distributed in the adult nervous system and their precursors have been reported to differentiate into astrocytes and even neurons in specific conditions (Rivers et al., 2008); however, their role in neuroinflammation is still poorly understood, as is that of ependymal and subependymal (Chi et al., 2006).

In addition to the death of specific neuronal populations, there are many other parallels between different neurodegenerative disorders. These include alteration of a diversity of neurotransmitters and intracellular signals, in particular of glutamate and calcium, which play key roles in excitotoxicity. Thus, alterations in cellular and molecular steady states give rise to changes that cannot be counteracted by the tissue, and lead to chronic and progressive neurodegenerative processes from which a return to normality is impossible. As advances in research are made, more similarities between these neurodegenerative diseases have been found on many different levels, from molecular to tissular.

1.2 Inflammation and neurodegeneration

The term neuroinflammation describes endogenous CNS tissue response to injury. Classically known as reactive gliosis, neuroinflammation refers to the aggressive response of glia to activating stimuli, analogous to the response of activated immune cells in peripheral tissues. Neuroinflammation has been associated with chronic CNS diseases such as multiple sclerosis, which is an unequivocal example of an inflammatory CNS disease. Other neurodegenerative diseases such as AD, ALS, PD, and HD lack the prominent infiltrates of blood-derived mononuclear cells that characterize autoimmune diseases. However, many substances involved in the promotion of inflammatory processes are present in the CNS of patients with such neurodegenerative diseases (Block et al., 2007).

Microglia are transformed and activated by a range of signals, such as neuronal death, mechanical injury and toxins (Block et al., 2007, Streit et al., 2004), and once activated they form the first line of defense against infection or injury to the CNS (Schwartz et al., 2006). Activated microglia acquire an amoeboid phenotype morphology similar to macrophages expressing the same markers, such as MHCI, MHCII, Iba1 and GLUT5 (Halliday & Stevens, 2011), and secrete proinflammatory molecules such as tumor necrosis factor-alpha (TNF- α), interferon γ , and interleukin 1 β ; they also upregulate oxidant molecules such as nitric oxide (NO) and O₂, which can protect against pathogens. This proinflammatory reaction eliminates hazards and repairs any damage. Microglia also release anti-inflammatory and trophic factors such as insulin-like growth factor 1 (IGF-1), interleukin 4, and interleukin 10, contributing to the repair and limitation of the inflammatory process (Block et al., 2007, Stoll et al., 2002). The proinflammatory or anti-inflammatory responses of the microglia are

influenced by surrounding astrocytes and inflammatory T-cell subsets, which can affect their phagocytic capacity and antigen-presenting cell properties.

All neurological disorders lead to activation of the microglia. Thus, microglial reaction represents the main mediator of the inflammatory process in neurodegenerative diseases, and microgliosis is directly related to the physiopathology of these. For example, microgliosis is associated with atypical and insoluble components caused by irregular protein folding and degradation pathways, altered subcellular localization, and the abnormal interactions with other cellular proteins found in AD, PD HD, Down syndrome and normal aging. Microgliosis is also associated with the formation of extracellular ionic precipitates, such as hydroxyapatites, which are frequently observed within the CNS areas involved in the disease (Rodriguez et al., 2009a, Saura et al., 1995), and is also present in encephalopathies caused by prions. This innate immune response is currently considered to be a potential pathogenic factor, since microglial reaction may engender neurodegenerative events, including amyloid-beta plaque formation, dystrophic neurite growth, and excessive tau phosphorylation.

1.3 Microglial reaction: Two sides of the same coin

In the healthy CNS, ramified resting microglia are active cells since they permanently scan their microenvironment (Wake et al., 2009). In response to any CNS injury or immunological stimuli, the microglia rapidly evolve from a surveillance state towards a more reactive one, through important phenotypical changes in response to activation signals released by the tissue (Schwartz et al., 2006). Microglia undergo a dramatic morphological transformation into amoeboid form and express an upregulated catalogue of molecules, such as CD14, major histocompatibility complex (MHC) molecules, chemokine receptors, CD11c, integrins, neurotrophins, and several other markers (Kettenmann et al., 2011). As such, reactive microglia can perform functions essential to neuron survival, such as phagocytosis to clear toxic and cellular debris, and innate immunity. Also involved in the release of trophic and anti-inflammatory factors, microglia facilitate repair through the guided migration of stem cells to the site of inflammation and injury.

In contrast, once microglia become overactivated they can produce detrimental effects through excessive production of a large array of cytotoxic factors, such as NO, TNF- α , reactive oxygen species, and pro-inflammatory cytokines (Lull & Block, 2010, Milligan & Watkins, 2009). Currently, the conditions that determine whether microglial reaction will be detrimental or beneficial to neuronal survival are poorly understood. However, it is becoming more widely accepted that although microglial activation is necessary and crucial for host defense and neuron survival, microglial overactivation leads to deleterious consequences.

Since every single microglial cell generates its own response to damage according to the nature and intensity of the signals released by the injured tissue, microglial cells do not constitute a homogenous cell population, but instead present a range of different phenotypes closely related to the evolution of the lesion process. In addition, some microglial cells become increasingly dysfunctional as they age, and may participate directly in the development of neurodegeneration (Block et al., 2007, Stoll et al., 2002). Microglia adopts a phenotype that mostly exacerbates tissue injury or promotes brain repair. Microglia can thus present two phenotypes, one of which is deleterious (also called M1 microglial phenotype) and the other benign (M2 microglial phenotype), depending on their intrinsic properties, interaction with the cellular microenvironment, and presence of

pathogenic factors (Halliday & Stevens, 2011, Henkel et al., 2009). Therefore, controlling microglial cell activation and the acquisition of positive or negative phenotypes is of major therapeutic interest in all CNS disorders related to neuroinflammation.

1.4 Astrocyte-microglia interactions: Who's the bad guy after all?

The classical view of astroglia, as simply presenting non-excitatory support to neurons, has changed radically in recent years. Astrocytes are now seen as elements that generate various local signals, including glutamate, to communicate with neurons and that influence the tissue outcome during neurodegeneration (Allaman et al., 2011). There is increasing evidence in support of this active role of astrocytes, suggesting that atypical astrocyte activation or astroglial dysfunction constitute maladaptive responses to brain injury that may feed the on-going pathologic process during neurodegeneration. For example, astrocyte dysfunction is a key factor in the pathogenesis of human neurological disorders (Seifert et al., 2006), and in the cognitive impairment of aged rats (Andrés et al., 2000). Furthermore, glutamate-induced chronic lesion in rat brain not only presents a lack of astrogliosis but also long term atrophy of astrocytes, suggesting a maladaptive response that may be a cause of the on-going pathologic process (Rodriguez et al., 2009a).

Moreover, astrocytes influence microglial behaviour (figure 1). For instance, astrocytes play a critical role in the activation of microglia under infectious conditions (Ovanesov et al., 2008). In addition, astroglial chemokines are involved in microglia/macrophage activation in multiple sclerosis with MCP-1/CCL2 and IP-10/CXCL10 directing reactive gliosis (Tanuma et al., 2006). Therefore, it is reasonable to assume that astrocytic activity can be influenced by microglial activation. Although a clear account of this dynamic relationship has yet to be proposed, the astrocyte-microglia interplay may determine the phenotype that microglial cells adopt during neurodegeneration.

Some findings have implicated astrocytes in chronic microgliosis, with a transition from an initial neuroprotective activity to a later cytotoxic one. TNF- α secretion is crucial for rapid autocrine microglial activation with both neuroprotective and cytotoxic effects, a process that is also fed by TNF- α released by reactive astrocytes (Suzumura et al., 2006). TNF- α actions leading to neuronal death or survival are dose dependent (Bernardino et al., 2008), since it can activate two specific receptors: TNFR1, with an intracellular death domain, and TNFR2, mainly involved in neuroprotection (Fontaine et al., 2002). Consequently, low concentrations of TNF- α would initially induce TNFR2-mediated neuroprotection, whereas a subsequent high concentration of TNF- α would be able to activate TNFR1 both in astrocytes and microglia and contribute to cell injury through the death domain of the receptor.

Astroglial S100 β is another of the factors that control microglial activity. Astrocytes release S100 β constitutively (Van Eldik & Wainwright, 2003) and increase this release upon stimulation by several factors, including TNF- α (Edwards & Robinson, 2006). Under normal conditions, released S100 β acts as a neurotrophic factor, countering the stimulatory effect of neurotoxins on (Reali et al., 2005) and stimulating astrocyte glutamate uptake (Tramontina et al., 2006). Released S100 β modifies astrocytic, neuronal and microglial activities, depending on the extracellular concentration of the former and the expression of the specific receptor for advanced glycation end-products (RAGE). At micromolar concentrations, S100 β upregulates IL-1 β and TNF- α expression in activated microglia via RAGE, with the requirement of concurrent activation of NF- κ B and AP-1 transcription factors (Bianchi et al., 2010). Furthermore, factors that modulate microglial reactivity, such as Ca²⁺ concentration,

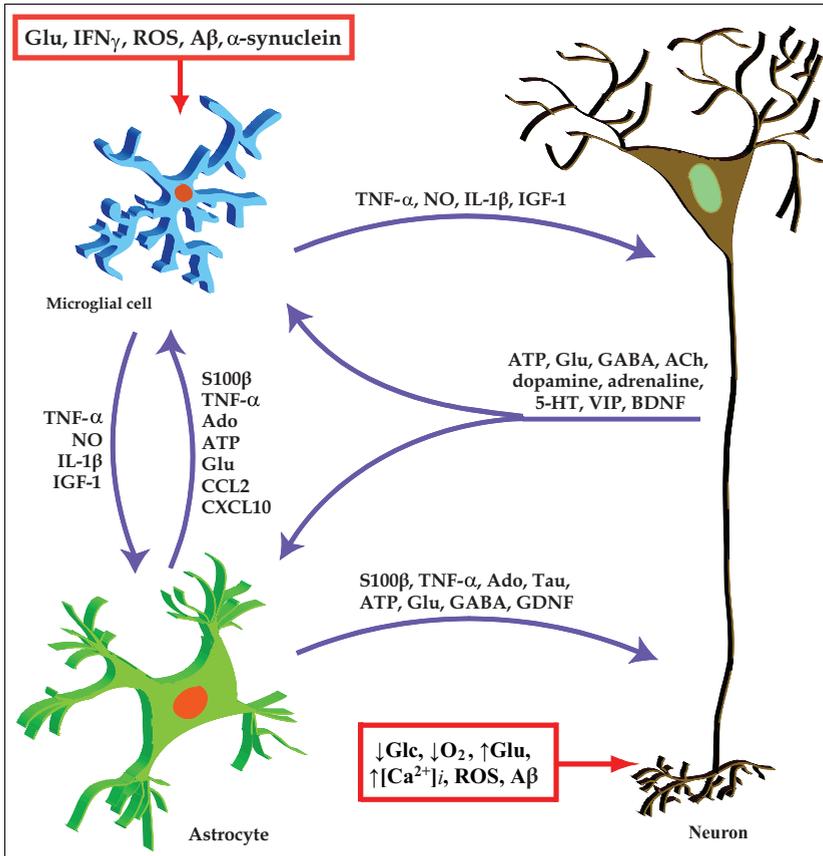


Fig. 1. Signalling systems in the microglia-astrocyte-neuron cross-talk. Astrocytes and microglia monitorize neuronal activity by sensing neurotransmitter release. In the same way, microglial cells have receptors to molecules released by astrocytes. Microglia integrate all these signals and release molecules that may modulate neuronal and astroglial activity. During neurodegeneration, changes in physiological parameters may trigger neuronal injury and/or microglial activation (molecules inside a red square) that modify those signal transduction systems. A β , amyloid beta; Ach, acetylcholine; Ado, adenosine; BDNF, Brain-derived neurotrophic factor; GABA, γ -aminobutyric acid; GDNF, Glial cell-derived neurotrophic factor; Glc, glucose; Glu, glutamate; 5-HT, serotonin; VIP, Vasoactive intestinal peptide; IFN γ , interferon gamma; ROS, reactive oxygen species; Tau, taurine

reactive oxygen species or TNF- α , also modify the RAGE response to S100 β (Edwards & Robinson, 2006) in a microglia-astroglia cross-talk that integrates these signaling systems. In contrast, at high concentrations S100 β binds the RAGE, which may mediate microglial activation during the course of brain damage (Bianchi et al., 2010). An increased release of S100 β during neurodegeneration (Li et al., 2011) will enhance inflammatory cytokine production and potentiate the switch of microglia to chronic cytotoxic activity, feeding the neurotoxic process and leading to neurodegeneration.

Thus, CNS neurodegeneration involves chronic microgliosis with a putative transition from an initial neuroprotective activity to a later cytotoxic one, with S100 β as a key modulator of microglial transition towards a cytotoxic response. This suggests a role for astrocytes in promoting the cytotoxic microglial phenotype through secretion of TNF- α , S100 β and other signals (Donato et al., 2009, Suzumura et al., 2006). In this scenario, the interplay between trophic, neuroprotective, inflammatory and cytotoxic functions of both cell types during brain injury will determine the evolution of the neurodegenerative process. Precise control of these processes thus requires a dynamic view of their interactions so as to allow effective development of approaches to neuroprotection.

2. CNS calcification and neurodegeneration

2.1 Enduring effects of excessive synaptic glutamate

Glutamate accounts for most of the excitatory synaptic activity in the CNS and has been implicated in learning, memory, synaptic plasticity and neurotrophic activity processes. Glutamate receptors have been classified into three groups: two ionotropic groups -N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-kainate receptors-; and one group of metabotropic receptors, which are coupled to G proteins. Although non-NMDA receptors are not initially permeable to Ca²⁺, glutamate release in the synaptic cleft increases post-synaptic and glial membrane permeability, leading to a transient increase in intracellular Ca²⁺ concentration ([Ca²⁺]_i) (Obrenovitch et al., 2000).

Excessive activation of glutamate receptors can trigger neuronal death through a process characterized by chronic glutamate release and the consequent [Ca²⁺]_i dys-homeostasis in neurons and astrocytes (Arundine & Tymianski, 2004) and formation of [Ca²⁺]_i precipitates, the size and number of which depends on the CNS area involved and CNS maturation (Bernal et al., 2000b, Rodríguez et al., 2001). This process, defined as excitotoxicity, also involves cellular influxes of Na⁺ and Cl⁻ and efflux of K⁺, with ensuing cell swelling (Chen et al., 1998, Katayama et al., 1995). Because of the complexity and diversity of the processes taking place at the glutamatergic synapse, any disturbance at the pre-synaptic, post-synaptic, or astroglial level may trigger a chronic excitotoxic process. For example, ALS presents a loss of selectivity of ionotropic receptors (Obrenovitch et al., 2000) and deficiencies in glial re-uptake of glutamate (Liévens et al., 2000). These glutamate-related dysfunctions contribute to explaining phenomena such as the aging-associated hypoactivity of NMDA receptors observed in AD (Olney et al., 1997), and the specific AMPA-receptor increment detected in the hippocampus of aged, cognitive-impaired rats (Le Jeune et al., 1996).

One of the consequences of excitotoxic-induced neuronal loss is the alteration of other neurotransmitter systems and neuromodulators. For example, long-term ibotenic-induced lesion in the basal forebrain of rat leads to a loss of cholinergic afferences and to decreased extracellular noradrenaline, glutamate, and taurine (Boatell et al., 1995). This cortical reduction in glutamatergic transmission presents a temporal pattern which, together with the development of Ca²⁺ precipitates and a decrease in the cholinergic and noradrenergic functions (Saura et al., 1995), mimics the neurochemical modifications described in AD. Similarly, one year after acute lesion, the cortical and hippocampal decrease in brain-derived neurotrophic factor, fibroblastic growth factor and glucocorticoid receptor, and the increase in c-fos expression in the septal area were still significant (Boatell et al., 1992). Thus,

excitotoxic lesions in basal forebrain can modify long-term cortical adaptive responses, and may modulate the expression of glutamate receptor. Some of these effects, such as the decrease in brain-derived neurotrophic factor and the increase in *c-fos* expression, also reflect the molecular alterations present in AD.

Given these toxic effects, adaptations that act to control glutamatergic neurotransmission and Ca^{2+} movements in the cell may be potentially protective. Over time, these defenses are developed to act at any time during an excitotoxic event, involve different cellular types such as neurons, astrocytes and microglia, and deal with the cellular and molecular mechanisms of glutamatergic neurotransmission. These mechanisms include defenses that: a) decrease neuronal excitability, b) decrease glutamate accumulation in the synapse, c) limit Ca^{2+} mobilization in the postsynaptic neuron and protect against calcium-dependent degenerative effects, and d) enhance neuronal energy (Rodriguez et al., 2009b, Sapolsky, 2001).

If the compensatory mechanisms are not sufficiently effective, the initial acute neuronal injury due to an increase in $[\text{Ca}^{2+}]_i$ leads, with time, to a chronic lesion. This secondary excitotoxicity appears in neurons after the massive entrance of Ca^{2+} and Na^+ through ionotropic glutamate receptors, an entrance that is supplemented by Ca^{2+} release from the endoplasmic reticulum following activation of mGluRs. As a result, an excessive $[\text{Ca}^{2+}]_i$ increment occurs (Verkhatsky, 2007), which activates the mechanisms triggering neuronal death. Ca^{2+} extrusion and buffering are activated when $[\text{Ca}^{2+}]_i$ increases (Mattson & Chan, 2003), with high expenditure of energy through Ca^{2+} -ATPases. (Figure 4).

Calcium homeostasis disturbances are present in all neurodegenerative disorders (Mattson, 2006). Dysregulation of Ca^{2+} homeostasis alters the rapid and coherent activation of neurons, and is therefore ultimately responsible for many aspects of brain dysfunction and CNS diseases. For example, an increased rate of Ca^{2+} -mediated apoptosis may cause neuronal death in the penumbra of cerebral ischemia, or may underlie the etiology of chronic neurodegenerative disorders such as PD and AD. Calcium precipitation that coincides with microglial activation, amyloid deposits and other ion accumulations in AD may thus be a key element of the neurodegenerative process (Ramonet et al., 2006).

2.2 Neurodegeneration as a result of disturbances in calcium homeostasis

Regulation of intraneuronal Ca^{2+} movements is a key element to ensure adequate cellular response at all times and produce a physiological effect. Ca^{2+} participates in most cellular functions, including membrane excitability and secretion, energy production, synaptic transmission, gene regulation and plasticity, cell proliferation and cell death (Arundine & Tymianski, 2003). As a consequence, cell function requires tight control of Ca^{2+} homeostasis between extra- and intracellular compartments at all times, including the production of sufficient energy from glycolysis to maintain different gradient concentrations such as a 1/10,000 inside-outside cell concentration (Verkhatsky, 2005). Most calcium within cells is sequestered in the mitochondria and the endoplasmic reticulum. Intracellular free calcium concentrations fluctuate widely, from roughly 100 nM to over 1 μM , due to release from cellular stores or influx from extracellular fluid. These fluctuations are integral to the role of calcium, and unless compensated for by some other mechanism, any dysregulation will have severe cellular consequences, the effects of which will be propagated to the surrounding cells, namely, neurons and glial cells (Beck et al., 2004). For example, activation of glutamate receptors by excitatory signals leads to a massive increase in cytoplasm Ca^{2+} levels, which in turn activates a cascade of events to produce a neuronal response. A return

to basal activity requires a considerable expenditure of energy to bring Ca^{2+} back to initial levels. Any failure in these multiple, coordinated steps will alter neuronal signalling and interfere with neuronal network functions (Rodriguez et al., 2009b).

Reduction of $[\text{Ca}^{2+}]_i$ involves a high mitochondrial intake of Ca^{2+} that may lead to loss of the mitochondrial membrane potential and the production of reactive oxygen species, thereby decreasing cellular respiratory capacity and ATP formation from ADP and Pi (Chan et al., 2007a). As a result, there is an acceleration of anaerobic glycolysis with a net lactate production that contributes to tissue acidification and progression of damage. Disturbances in Ca^{2+} homeostasis in astrocytes reduce neuronal support, in particular through alteration of the glutamate/glutamine cycle and a reduction of glucose delivery to neurons (Pellerin et al., 2007, Ramonet et al., 2004) (Figure 4). Consequently, astrocyte dysfunction can lead to increased synaptic glutamate levels and glutamate receptor overactivation, combined with reduced neuronal energy, resulting in neuronal damage. Under these conditions of high Ca^{2+} and Pi and low ATP, formation of hydroxyapatite precipitates to reduce Ca^{2+} cytoplasm activity at low energy costs can occur in neurons and astrocytes (Rodriguez et al., 2000). This new step in calcium homeostasis temporarily helps the cell to resist excessive stimulatory signals and return to basal activity by dissolving paracrystal elements. However, in most cases, the hydroxyapatite crystals show progressive growth and participate in cell death and CNS damage.

Depending on the importance of the damage, microglial activation may take place, initially expressing neuroprotective signals to help avoid further neuronal death, but then changing progressively to an inflammatory phenotype (Graeber, 2010). Glycolysis, highly stimulated in microglia to ensure and maintain their activated stage, reduces neuronal glucose and oxygen availability. Consequently, microglial participation in neuronal death includes not only neuroinflammation, but also reduction in neuronal energy availability (Allaman et al., 2011). If, as asserted by Gyuri Buzsaki in *Rhythms of the Brain* (Buzsáki, 2006), “Brains are foretelling devices and their predictive powers emerge from the various rhythms they perpetually generate”, any significant alteration of the neuronal rhythms caused by an abnormally high Ca^{2+} concentration in neurons or glia should alter the brain’s ability to pause, adapt and learn, and can lead to disease. This is the case of neurodegenerative diseases, which exhibit diverse clinical and neuropathological phenotypes but share the common feature of progressively reduced cell function and survival within the nervous system, leading to neurological disability and often death. As such, several different CNS disorders can be induced following the same injury, due to the multi-directional interactions between the neurons, glial cells, extracellular matrix, endothelia and host immune cells that regulate tissue homeostasis and orchestrate neuroinflammation and degeneration. Furthermore, the characteristics of each neuronal population and network, the different gliopathic changes occurring between CNS areas, the various microglia phenotypes and the abundance and distribution of glutamate receptor subtypes and of Ca^{2+} -binding proteins, all participate directly in the properties of the neurodegenerative parameters (Graeber & Streit, 2010, Rodriguez et al., 2004, Rodriguez et al., 2009a) that will determine the dynamics and progression of the disease in the specific affected areas. For example, PD and AD are both regarded as diseases that are initiated by neuronal death to which the immune system responds, as evidenced by astroglial and microglial activation with pathogenic consequences (Agostinho et al., 2010, Halliday & Stevens, 2011). Similarly, multiple sclerosis is typically considered a neuroinflammatory disorder, but one in which neuronal injury plays an active role in

regulating neuroinflammation, as has been recently reported (Haider et al., 2011). In all of these diseases, dysregulation of Ca^{2+} homeostasis has been considered a pathophysiological factor linked to neuronal degeneration, and the formation of intracellular Ca^{2+} deposits with different characteristics as regards size and distribution - reflecting differential CNS area vulnerability - has frequently been reported (Hashimoto et al., 2003, Ramonet et al., 2006).

2.3 CNS calcification

In immature human CNS, Ca^{2+} -mediated excitotoxicity is associated with a calcification process that directly correlates with neuronal loss and the extent of injury. Revealed by Alizarin red staining and appearing in TEM and X-ray microanalyses of animal neurodegeneration models, small and large intracellular Ca^{2+} precipitates indicate the formation of a paracrystalline structure of hydroxyapatites localized within neurons and astrocytes (figure 2).

Glutamate analog microinjection in rat CNS leads to an intracellular Ca^{2+} precipitation similar to brain calcification in humans (Ramonet et al., 2006, 2002). As these Ca^{2+} deposits can be observed in several areas of rat brain after microinjection of different excitotoxins (Bernal et al., 2000b, Rodriguez et al., 2000, Saura et al., 1995), their formation does not depend on the glutamate receptor subtype initially stimulated. However, their size, number and distribution vary with both the activated receptor and the CNS area. For example, sensitivity to AMPA-induced calcification decreased from the globus pallidus, cerebral cortex, hippocampus, medial septum, to retina (Rodriguez et al., 2000). Moreover, in medial septum, the degeneration associated with microinjection of ibotenic and quisqualic acids was characterized by significant atrophy and no calcification (Mahy et al., 1996, Saura et al., 1995). In similar conditions, AMPA microinjection resulted in similar atrophy and Ca^{2+} deposits at the injection site (Rodriguez et al., 2009a).

Ca^{2+} deposits do not occur in all cells that degenerate in response to excitotoxins. For example, in the basal forebrain and medial septum, the calcification observed in GABAergic cells was not detected in cholinergic neurons. The former, together with astrocytes, seem to participate actively in the calcification process (Mahy et al., 1999). Differences in the neuronal phenotype of Ca^{2+} buffering and extrusion systems, specific energy needs, expression of the glutamate subtype receptor and different astroglial populations, should explain this variability. The ultrastructural study of tissue affected by excitotoxicity has also contributed to our understanding of calcification. Ca^{2+} deposits within hypertrophied astrocytes have been characterized in the basal forebrain and hippocampus which ranged from 0.5 to 10 μm in diameter and were formed by numerous, small, needle-shaped crystals associated with cellular organelles, such as microtubules, cisternae, vesicles or mitochondria, with no signs of neurodegeneration (figure 2). Larger inclusions were surrounded by reactive microglia, a finding that was also observed in tissue after specific localization by *in vitro* autoradiography (Bernal et al., 2000b, Petegnief et al., 1999). X-ray microanalysis has shown an electron-diffraction ring pattern characteristic of a crystalline structure similar to apatites (Kim, 1995), and a Ca/P ratio of 1.3 ± 0.2 of cytoplasmic deposits (Figure 2). This ratio, lower than the theoretical apatite value of 1.67, is also typical of biological crystals, which do not present an ideal organization (Rodriguez et al., 2000). As biological hydroxyapatites, these deposits are similar to those observed in several human peripheral nervous system tissues (Kodaka et al., 1994).

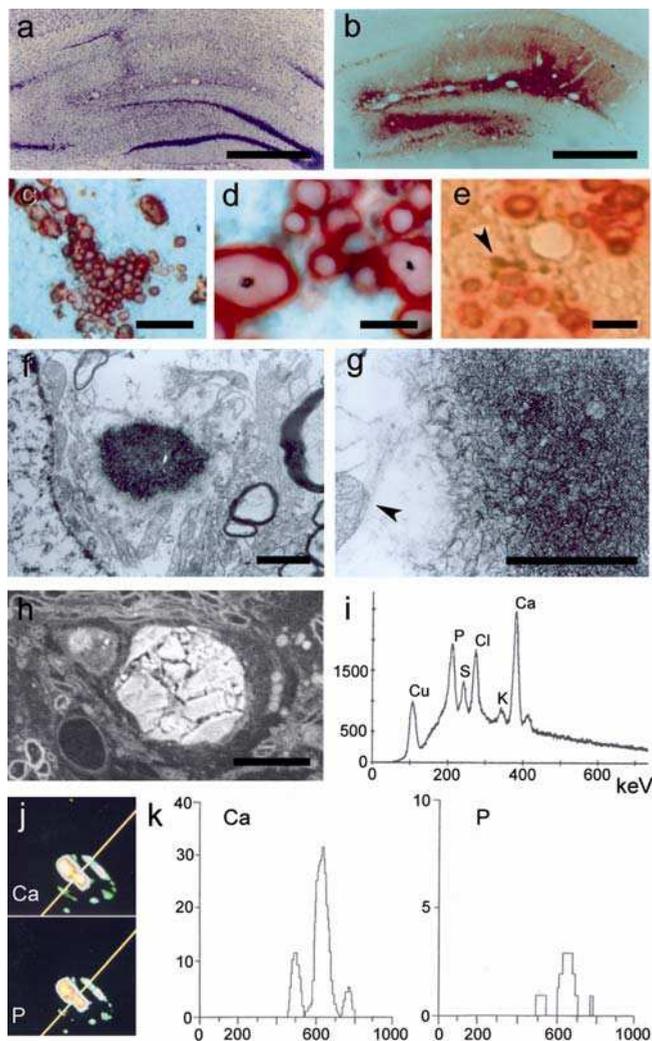


Fig. 2. Characterization of calcium deposits induced by ibotenic acid in the rat brain. Microphotographs of a) Nissl stained section of a rat hippocampus 15 days after the injection and b) Alizarin red stained section of the same hippocampus showing calcium deposits associated with the lesion. c-d) calcium deposits showed different sizes in the rat globus pallidus 2 months after injection. e) Isolectin B4 histochemistry (brown staining) counterstained with alizarin red showing the microglial reaction (arrowhead) associated with calcium deposits. f) Hypertrophic astrocyte with an intracytoplasmic calcium deposit by TEM. g) Detail of the ultrastructure of calcium deposit within an astrocyte. Note the normal appearance of the surrounding mitochondria (arrowhead). X-ray image h) and spectrum analysis i) of one calcium deposit in a non-osmificated sample with a calculated Ca/P ratio of 1.3. False colour X-ray image mapping j) and distribution plots k) of Ca and P of the same deposit. Bars: a-b, 1 mm; c, 100 μm ; d-e, 20 μm ; f, 0.6 μm , g, 0.2 μm ; h, 10 μm

Experimental models of bone formation (i.e. hydroxyapatite formation *in vitro*) (Andre-Frei et al., 2000) have shown that rather than Ca^{2+} , a minimal amount of phosphorus, as inorganic phosphate, is crucial for crystal nucleation in a collagen matrix. Similarly, organic phosphate residues of the phosphoproteins also play a direct and significant role in the process of *in vitro* nucleation of apatite by bone collagen, whereas collagen itself does not promote the precipitation of Ca^{2+} or phosphate (Andre-Frei et al., 2000). Therefore, excitotoxicity-induced calcification in the rat brain depends on an increase in intracellular inorganic phosphate (i.e. ATP depletion) and, most importantly, on the degree of protein phosphorylation. Thus, the Ca^{2+} -binding-protein-dependent kinases and activity of the neurotrophic factor ultimately determine calcification.

In aqueous solutions, hydroxyapatite crystallization takes place in two sequential steps (Barat et al., 2011): in the first, crystal nucleation occurs spontaneously with subsequent growth to some nanometers, when phosphate and Ca^{2+} ions reach a certain concentration; in the second step, an accretion process of these nanocrystals on a proteinic net takes place until reaching a maximum size of 20 micrometers. While the first process facilitates re-solubilization of the crystal, the second produces a stable precipitate and requires a catalytic agent. These two mechanisms may help explain the size differences we found between several areas of the CNS. For example, the large insoluble Ca^{2+} precipitates (mean size 20 μm) found after AMPA microinjection in globus pallidus (Petegnief et al., 1999) fit well with the second step theory, whereas the small deposits (mean size lower than 3 μm) obtained in hippocampus after the same procedure (Rodríguez et al., 2004) may reflect the lack of a catalytic agent for accretion, or an equilibrium between crystal formation and solubilization. Furthermore, blockade of glial glutamate uptake in rat striatum (Liévens et al., 2000) produced a spherical lesion with a central necrotic core surrounded by a penumbra zone similar to that caused by focal ischemia. Three days after treatment, an astroglial reaction and small Ca^{2+} deposits (mean diameter < 1 μm) were observed in the penumbra area. Eleven days later, these deposits had disappeared, the penumbra zone had recovered from injury and the necrotic area was partially repaired (Liévens et al., 2000). In this scenario, compensatory mechanisms helped normalize Ca^{2+} homeostasis and avoid further neuronal death. The tissue recovered the ability to use extrusion mechanisms, and re-solubilization of Ca^{2+} precipitates took place.

When Ca^{2+} deposits are localized extracellularly due to cell death, a microglial reaction is activated for their phagocytic removal. This microglial reaction also participates in the neuronal death seen in chronic neurodegenerative processes, but is dissociated from astrogliosis. In some animal models of neurodegeneration, a recovery has been observed associated with the disappearance of Ca^{2+} deposits. In other excitotoxic rodent models, the on-going neurodegenerative process increased with time and the Ca^{2+} deposits remained present, associated with microglial reaction.

2.4 The calcification process and ageing

In the neonate mammalian brain, considered more resistant to hypoxia-ischemia than adult CNS, dysregulation of Ca^{2+} homeostasis together with lactate acidosis are considered the main factors causing neuronal death. As premature-neonates are more resistant to hypoxia-ischemia than term neonates, we studied the relationship between differences in human brain vulnerability to hypoxia-ischemia during the perinatal period and brain calcification in the basal ganglia, cerebral cortex, and hippocampus (Rodríguez et al., 2001). The number

and size of the observed non-arteriosclerotic calcifications were area-specific and increased in term neonates (Figure 3). The basal ganglia presented the highest degree of calcification and the hippocampus the lowest, mainly in the CA1 subfield. In all cases, neuronal damage was associated with astroglial reaction and Ca^{2+} precipitates, with microglial reaction absent in the hippocampus. These data are consistent with those obtained following long-term excitotoxic lesions in adult rat brain and support the involvement of excitotoxic processes in hypoxia-ischemia damage.

A comparison between lifespan and degree of calcification (Figure 3) demonstrated that in all cases, highest calcification occurred within two months of hypoxia-ischemia, and that semi-calcification time was very short (less than 10 days). Independent of subjective measurements, this last parameter suggests that calcification depends on the degree of brain differentiation and initial cerebral injury, but not on the time-course of the lesion. Moreover, the mechanisms leading to Ca^{2+} precipitation seem to be similar for all brain areas. If this is true, neurons of each CNS structure degenerate through a common mechanism, which is linked to disturbances in Ca^{2+} homeostasis. As each area of the brain participates in specific physiological functions, the resultant pathology will depend on the specific neuronal death of the area affected.

Aging increases neuronal vulnerability to toxic compounds, including drugs that impair energy metabolism and induce secondary excitotoxic processes (Brouillet et al., 1993). However, a decreased susceptibility of aged rats to excitotoxins such as quinolinic or kainic acids has been reported (Kesslak et al., 1995). AMPA-induced Ca^{2+} deposits in rat hippocampus are age-dependent, since young rats (3 months old) present greater areas of calcification than middle-aged ones (15 months old) (Bernal et al., 2000a). In this study, glial reaction, γ -aminobutyric acid (GABA)-uptake activity and immunostaining of Ca^{2+} binding proteins showed the same response. Therefore, the vulnerability of hippocampal neurons to AMPA-induced neurodegeneration decreases with age between 3 and 15 months. Similar results have been found in other brain areas, such as the striatum and the nucleus basalis magnocellularis. This reduced vulnerability may be related to several factors: for example, age-associated variations in the relative abundance of glutamate receptors and pre-synaptic alterations of glutamate release may explain, at least in part, an increased resistance to excitotoxicity in the hippocampus (Mullany et al., 1996, Nicolle et al., 1996).

This effect is compatible with the increased vulnerability to excitotoxicity observed in the oldest animals (Brouillet et al., 1993), since some of the factors responsible for injury resistance may follow a biphasic pattern, with a progressive increase until reaching maturity followed by a subsequent decrease (Coleman et al., 1990). Many authors have also described biphasic variations in several parameters during aging, with an opposite tendency before and after middle age (Villa et al., 1994). We observed a biphasic variation in monoamine oxidase B (MAO-B) during aging in most human brain areas: up to the age of 50-60 years old, MAO-B levels remain constant, but start to increase thereafter (Saura et al., 1997). This finding may be due to the presence of MAO-B rich reactive astrocytes in response to neuronal degeneration. A similar increase in plaque-associated astrocytes has been found in patients with AD (Saura et al., 1994). As MAO-B activity is associated with reactive oxygen species production, astrocytes may contribute to the age-associated decline of neurological functions. The evidence that an increase in AMPA receptor correlates negatively with MAO-B in age-associated learning-impaired rats also suggests that a gliopathic reaction may be involved in neuronal dysfunction (Andrés et al., 2000).

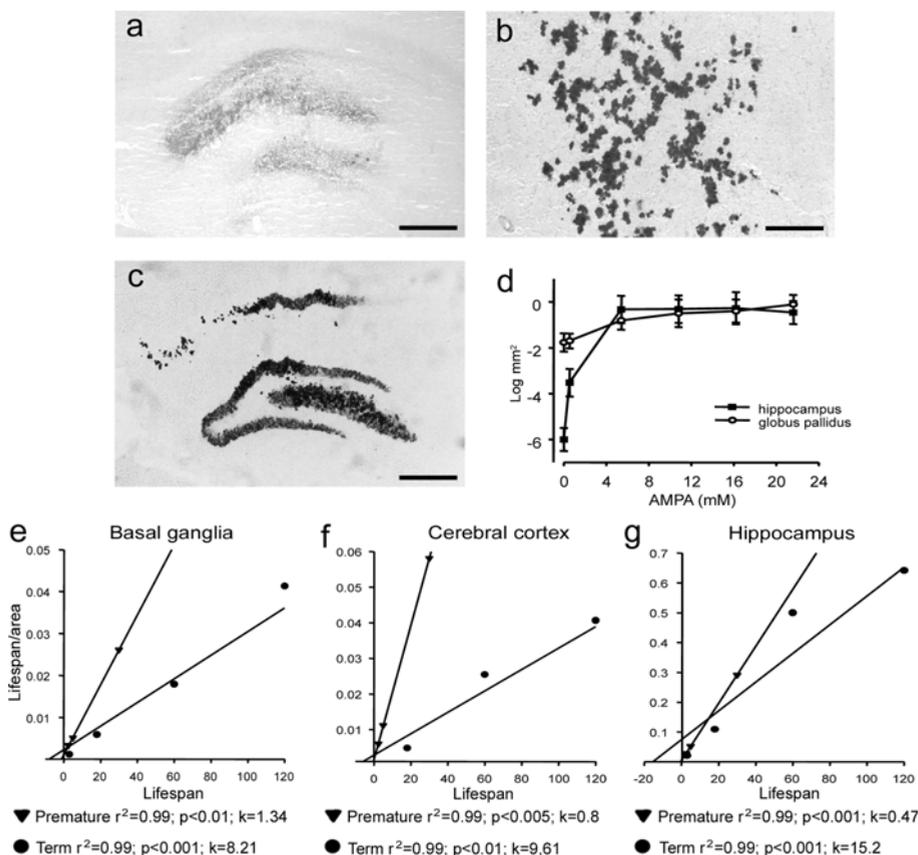


Fig. 3. Calcification depends on the brain area but also on the glutamate receptor involved. a) AMPA induces small calcium deposits in the rat hippocampus, affecting mainly the CA1 radiatum and lacunosum moleculare subfields. b) AMPA microinjection in the globus pallidus induces larger calcium deposits. c) NMDA microinjection in the hippocampus induces the formation of large calcium deposits located mainly in the pyramidal CA1 and granular dentate gyrus. d) The plots show comparison of the AMPA dose-response study in the hippocampus and the globus pallidus. e-f) Correlation plots of the hypoxia ischemia-induced calcification in the basal ganglia e), cerebral cortex f) and hippocampus g) of premature and term neonates. Calcification was calculated in a representative area (1 mm^2) and the lifespan corresponds to the time of injury in days. k , days to reach half of the maximal calcified area. Bars, $300 \mu\text{m}$

Thus, the correlation between the calcification process, neuronal loss and the extent of CNS injury disappears with aging, but differences in CNS area vulnerability to calcification are maintained. The components that underlie the specific vulnerability of each brain area are thus already expressed in human neonates. The permanent area differences are associated with significant variations in the response to specific Ca^{2+} channel blockers such as nimodipine and TMB-8 (Bernal et al., 2009, Petegnief et al., 2004), and illustrate the functional diversity of each area and the difficulty encountered in ensuring the efficacy of

such types of treatment. Similar results concerning differences between calcium precipitates and brain area susceptibility have been observed in congenital toxoplasmosis (Safadi et al., 2003, Surendrababu et al., 2006). Cerebral calcification has been described in 65% of these patients, with calcified foci distributed predominantly in the cortex in the form of tiny flecks, and as linear streaks in the basal ganglia.

The calcification process can thus be considered a new stage in cytoplasmic calcium homeostasis taking place in a diversity of CNS injuries to reduce calcium signalling at no energy cost. When located extracellularly due to cell-death, these precipitates activate a permanent microglial reaction aimed at their removal but which rapidly turns into chronic damage and aggravation of neurodegeneration.

2.5 Uncoupling of retaliatory systems and energy availability

The balance between retaliatory system actions and energy metabolism constitutes a fine equilibrium in physiological conditions, but it can be disrupted by glutamate-mediated neuronal injury to then participate in the evoked neurodegenerative process. For example, AMPA-microinjection in medial septum induces a progressive cholinergic and GABAergic loss associated with a long-term decline of the hippocampal functions and decreased glutamatergic activity (Rodriguez et al., 2005). Other effects of this lesion imply modifications of adenosine and taurine transmissions, glutamate recycling and glucose metabolism (Ramonet et al., 2004, Rodriguez et al., 2005). Over time, adenosine replaces GABA functions to avoid further excitotoxic damage when cholinergic and GABAergic processes are compromised.

Long-term septal lesion-induced neuronal loss in the hippocampus is apoptotic, with enhancement of neuronal glycolysis. Together with the cleavage of caspase 3, a glutamate-glutamine cycle displacement towards glutamine production reduces glutamate synthesis (Ramonet et al., 2004). In addition, synaptic glutamine is decreased, probably through expulsion to vessels, where it exerts a vasodilatory effect through NO synthesis inhibition (Mates et al., 2002). In this scenario, the reduction in glutamate signaling and increased neuronal energy metabolism both reflect a neurodegenerative process with deficient adaptation of the retaliatory systems and a chronic energy requirement to execute the apoptotic program.

This chronic energy requirement induces mitochondrial damage, in turn leading to acidosis in cells and the extracellular space (Hertz, 2008). Mitochondrial damage forces the cell to shift from an aerobic to an anaerobic metabolism, and as a result lactate is produced with the formation of two ATPs and the release of two protons. After trauma and ischemia, extracellular lactate increases dramatically and pH decreases. To ensure neuronal viability during and even after human hypoxia, glial glucose is only oxidized to lactate, which is rapidly transported into neurons for its complete oxidation (Sibson et al., 1998). In parallel, a Ca^{2+} influx causes rapid cytoplasmic acidification (Verkhatsky, 2007) through: a) activation of membrane Na^+/H^+ exchanger to restore the Na^+ gradient, and b) the Ca^{2+} -dependent displacement of protons bound to cytoplasmic anions (Arundine & Tymianski, 2004). Furthermore, H^+ also appears during some chemical reactions, such as phospholipid hydrolysis.

2.6 Functional relevance of the calcification process

The massive astroglial production of lactate to help compensate for the neuronal energy depletion caused by excitotoxicity is a key factor in brain calcification (Figure 4). pH

reduction associated with increased lactate concentration facilitates solubility of Ca^{2+} and the formation of H_2PO_4^- , HPO_4^{2-} and PO_4^{3-} ions from inorganic phosphate (Rodríguez et al., 2000) and phosphorylated proteins. Because of the very high $\text{Ca}^{2+} / \text{H}_2\text{PO}_4^-$, HPO_4^{2-} , PO_4^{3-} affinity, apatite nucleation may occur, with the subsequent growth of crystalline formations together with neurodegeneration. In this case, calcification of each lesioned area will also depend on phosphate availability and the differential capacity of glial cells to release lactate during degeneration.

Wide variations have been described in the extent of calcification in pathological cases (Ramonet et al., 2002, Rodríguez et al., 2001) and animal species (Ramonet et al., 2006). However, the homogeneous morphology of these deposits suggests common synaptic processes (Ramonet et al., 2006) where variability depends on cellular type (astrocyte or neuron), glutamatergic activity, and energy availability (Figure 4). These factors modify Ca^{2+} homeostasis and may trigger cellular calcification through a common mechanism (Ramonet et al., 2006). Thus, hydroxyapatite formation, with the subsequent reduction of free Ca^{2+} ions, may take place as an alternative homeostatic step to reduce excitotoxicity (Ramonet et al., 2006, Rodríguez et al., 2000), and a number of findings lend support to this interpretation. For example, it has been observed that mitochondria close to Ca^{2+} deposits appear normal at electron microscopy level (Mahy et al., 1999, Rodríguez et al., 2000), despite

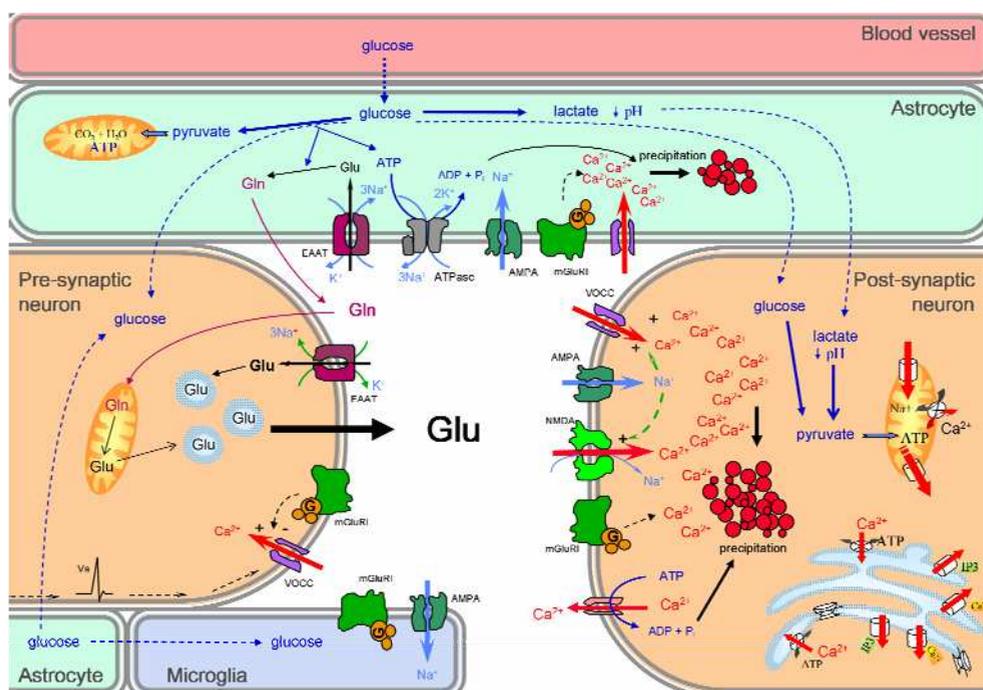


Fig. 4. Excitotoxicity modifies cell calcium homeostasis in the brain. Drawing of the excitotoxic process induced by glutamate, with the intercellular precipitation of calcium as part of the calcium homeostasis. The metabolic pathway of lactate with the communication between endothelial, astroglial, microglial and neuronal compartments is included in the diagram

the fact that mitochondrial dysfunction constitutes a primary event in NMDA-induced degeneration (Schinder et al., 1996). This hypothesis is also consistent with the finding that neurons undergoing prolonged stimulation of NMDA receptors can survive in the presence of $[Ca^{2+}]_i$ chelators. Very high levels of cytoplasmic Ca^{2+} are not necessarily neurotoxic, and an effective uptake of this element into mitochondria is required to trigger NMDA-receptor-stimulated neuronal death (Stout et al., 1998). Moreover, in rat globus pallidus, an AMPA-dose-response study has shown a dose-dependent increase in calcification, which was not accompanied by an increase in astrogliosis (Petegnief et al., 1999). In the hippocampus, AMPA induced a calcified area larger than the injured area. In this same structure, the selective adenosine-A2a-receptor antagonist 8-(3-chlorostyryl)-caffeine increased NMDA-induced neuronal loss while calcification was decreased (Robledo et al., 1999). All these data indicate that Ca^{2+} precipitation does not necessarily reflect neuronal death and that, as proposed for retinal excitotoxic damage (Chen et al., 1999), in addition to Ca^{2+} other factors such as Na^+ and Cl^- influx, cell swelling and acidosis induce excitotoxic neuronal damage.

3. Conclusions

Neurodegenerative disorders are characterized by the appearance of distinct neurodegenerative parameters that determine the induction of a chronic process with underlying glutamate-mediated excitotoxicity and Ca^{2+} dys-homeostasis. At tissue level, the pathogenesis of each disorder depends on the neuronal type involved, synaptic density, glial interactions, and vicinity of vascularization. For each neuron, astrocyte and microglial type, the group of glutamate and cytokine receptors, the Ca^{2+} binding protein content, protein phosphorylation levels, and all elements that participate in energy needs and glucose availability will constitute the factors involved in the appearance of the lesion. In this scenario, CNS calcification can be considered one of the few common mechanisms already available at an early age to help buffer disturbances in Ca^{2+} homeostasis at no energy cost. Over time, CNS maturation includes a massive increase in synaptic connections, the organization of inhibitory systems and greater cellular complexity. As it becomes more sophisticated, the CNS relies on a greater diversity of mechanisms to prevent CNS injury. This would explain why calcification is observed in neurodegenerative diseases, but does not correlate with CNS damage.

4. Acknowledgements

This research was supported by grants SAF2008-01902 and IPT-010000-2010-35 from the Ministerio de Ciencia e Innovación, and by grant 2009SGR1380 from the Generalitat de Catalunya, Spain.

5. References

- Agostinho, P., Cunha, R.A. & Oliveira, C. (2010). Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. *Curr Pharm Des*, Vol. 16, No. 25, pp. 2766-78, ISSN 1873-4286
- Allaman, I., Belanger, M. & Magistretti, P.J. (2011). Astrocyte-neuron metabolic relationships: for better and for worse. *Trends Neurosci*, Vol. 34, No. 2, pp. 76-87, ISSN 1878-108X

- Andre-Frei, V., Chevally, B., Orly, I., Boundeulle, M., Huc, A. & Herbage, D. (2000). Acellular mineral deposition in collagen-based biomaterials incubated in cell culture media. *Calc Tissue Int*, Vol. 66, No. pp. 204-211, ISSN 1432-0827
- Andrés, N., Rodríguez, M.J., Andrade, C., Rowe, W., Quirion, R. & Mahy, N. (2000). Increase in AMPA receptors in aged memory-impaired rats is not associated with increase in monoamine oxidase B levels. *Neuroscience*, Vol. 101, No. 4, pp. 807-810, ISSN 0306-4522
- Arundine, M. & Tymianski, M. (2003). Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. *Cell Calcium*, Vol. 34, No. 4-5, pp. 325-337, ISSN 0143-4160
- Arundine, M. & Tymianski, M. (2004). Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. *Cell Mol Life Sci*, Vol. 61 No. 6, pp. 657-668, ISSN 1420-682X
- Barat, R., Montoya, T., Seco, A. & Ferrer, J. (2011). Modelling biological and chemically induced precipitation of calcium phosphate in enhanced biological phosphorus removal systems. *Water Res*, Vol. 45, No. 12, pp. 3744-52, ISSN 1879-2448
- Beck, A., Nieden, R.Z., Scheinder, H.P. & Deitmer, J.W. (2004). Calcium release from intracellular stores in rodent astrocytes and neurons in situ. *Cell Calcium*, Vol. 35 No. pp. 47-58, ISSN 0143-4160
- Beers, D.R., Henkel, J.S., Zhao, W., Wang, J., Huang, A., Wen, S., Liao, B. & Appel, S.H. (2011). Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. *Brain*, Vol. 134, No. Pt 5, pp. 1293-314, ISSN 1460-2156
- Bernal, F., Andrés, N., Samuel, D., Kerkerian-Legoff, L. & Mahy, N. (2000a). Age-related resistance to α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-induced hippocampal lesion. *Hippocampus*, Vol. 10, No. pp. 296-304, ISSN 1098-1063
- Bernal, F., Petegnief, V., Rodriguez, M.J., Ursu, G., Pugliese, M. & Mahy, N. (2009). Nimodipine inhibits TMB-8 potentiation of AMPA-induced hippocampal neurodegeneration. *J Neurosci Res*, Vol. 87, No. 5, pp. 1240-1249, ISSN 0360-4012
- Bernal, F., Saura, J., Ojuel, J. & Mahy, N. (2000b). Differential vulnerability of hippocampus, basal ganglia and prefrontal cortex to long-term NMDA excitotoxicity. *Exp Neurol*, Vol. 161, No. pp. 686-695, ISSN 0014-4886
- Bernardino, L., Agasse, F., Silva, B., Ferreira, R., Grade, S. & Malva, J.O. (2008). Tumor necrosis factor-alpha modulates survival, proliferation, and neuronal differentiation in neonatal subventricular zone cell cultures. *Stem Cells*, Vol. 26, No. 9, pp. 2361-2371, ISSN 1066-5099
- Bianchi, R., Giambanco, I. & Donato, R. (2010). S100B/RAGE-dependent activation of microglia via NF-kappaB and AP-1 Co-regulation of COX-2 expression by S100B, IL-1beta and TNF-alpha. *Neurobiol Aging*, Vol. 31, No. 4, pp. 665-77, ISSN 1558-1497
- Block, M.L., Zecca, L. & Hong, J.S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*, Vol. 8, No. pp. 57-69, ISSN 1471-003X
- Boatell, M.L., Bendahan, G. & Mahy, N. (1995). Time-related cortical amino acid changes after basal forebrain lesion: a microdialysis study. *J Neurochem*, Vol. 64, No. 1, pp. 285-291, ISSN 0022-3042
- Boatell, M.L., Lindefors, N., Ballarín, M., Ernforms, P., Mahy, N. & Persson, H. (1992). Activation of basal forebrain cholinergic neurons differentially regulates brain-derived neurotrophic factor mRNA expression in different projection areas. *Neurosci Letters*, Vol. 136, No. pp. 203-208, ISSN 0304-3940

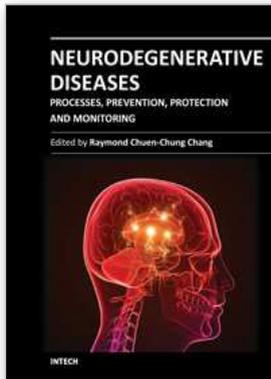
- Brouillet, E., Jenkins, B.G., Hyman, B.T., Ferrante, R.J., Kowall, N.W., Srivastava, R., Roy, D.S., Rosen, B.R. & Beal, M.F. (1993). Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. *J Neurochem*, Vol. 60, No. pp. 356-359, ISSN 0022-3042
- Buzsáki, G. (2006). *Rhythms of the brain* Oxford University Press, ISBN 0195301064, Oxford
- Chan, S.L., Liu, D., Kyriazis, G.A., Bagsiyao, P., Ouyang, X. & Mattson, M.P. (2007a). Mitochondrial uncoupling protein-4 regulates calcium homeostasis and sensitivity to store-depletion-induced apoptosis in neural cells. *J Biol Chem*, Vol. 281, No. 49 pp. 3739-37403, ISSN 0021-9258
- Chan, W.Y., Kohsaka, S. & Rezaie, P. (2007b). The origin and cell lineage of microglia: new concepts. *Brain Res Rev*, Vol. 53, No. 2, pp. 344-54, ISSN 0165-0173
- Chen, Q., Moulder, K., Tenkova, T., Hardy, K., Olney, J.W. & Romano, C. (1999). Excitotoxic cell death dependent on inhibitory receptor activation. *Exp Neurol*, Vol. 160, No. pp. 215-225, ISSN 0014-4886
- Chen, Q., Olney, J.W., Lukasiewicz, P.D., Almlı, T. & Romano, C. (1998). Ca²⁺-independent excitotoxic neurodegeneration in isolated retina, an intact neural net: a role for Cl⁻ and inhibitory transmitters. *Mol Pharmacol*, Vol. 53, No. 3, pp. 564-572, ISSN 0026-895X
- Chi, L., Ke, Y., Luo, C., Li, B., Gozal, D., Kalyanaraman, B. & Liu, R. (2006). Motor neuron degeneration promotes neural progenitor cell proliferation, migration, and neurogenesis in the spinal cords of amyotrophic lateral sclerosis mice. *Stem Cells*, Vol. 24, No. 1, pp. 34-43, ISSN 1066-5099
- Coleman, P., Finch, C. & Joseph, J. (1990). The need for multiple time points in aging studies. *Neurobiol Aging*, Vol. 11, No. pp. 1-2, ISSN 0197-4580
- Donato, R., Sorci, G., Riuzzi, F., Arcuri, C., Bianchi, R., Brozzi, F., Tubaro, C. & Giambanco, I. (2009). S100B's double life: intracellular regulator and extracellular signal. *Biochim Biophys Acta*, Vol. 1793, No. 6, pp. 1008-1022, ISSN 0006-3002
- Edwards, M.M. & Robinson, S.R. (2006). TNF alpha affects the expression of GFAP and S100B: implications for Alzheimer's disease. *J Neural Transm*, Vol. 113, No. 11, pp. 1709-15, ISSN 0300-9564
- Farina, C., Aloisi, F. & Meinl, E. (2007). Astrocytes are active players in cerebral innate immunity. *Trends Immunol*, Vol. 28, No. 3, pp. 138-45, ISSN 1471-4906
- Fontaine, V., Mohand-Said, S., Hanoteau, N., Fuchs, C., Pflizenmaier, K. & Eisel, U. (2002). Neurodegenerative and neuroprotective effects of tumor Necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. *J Neurosci*, Vol. 22, No. 7, pp. RC216, ISSN 1529-2401
- Graeber, M.B. (2010). Changing face of microglia. *Science*, Vol. 330, No. 6005, pp. 783-8, ISSN 1095-9203
- Graeber, M.B. & Streit, W.J. (2010). Microglia: biology and pathology. *Acta Neuropathol*, Vol. 119, No. 1, pp. 89-105, ISSN 1432-0533
- Haider, L., Fischer, M.T., Frischer, J.M., Bauer, J., Hoftberger, R., Botond, G., Esterbauer, H., Binder, C.J., Witztum, J.L. & Lassmann, H. (2011). Oxidative damage in multiple sclerosis lesions. *Brain*, Vol. 134, No. Pt 7, pp. 1914-24, ISSN 1460-2156
- Halliday, G.M. & Stevens, C.H. (2011). Glia: initiators and progressors of pathology in Parkinson's disease. *Mov Disord*, Vol. 26, No. 1, pp. 6-17, ISSN 1531-8257
- Hashimoto, N., Takeuchi, T., Ishihara, R., Ukai, K., Kobayashi, H., Iwata, H., Iwai, K., Mizuno, Y., Yamaguchi, H. & Shibayama, H. (2003). Glial fibrillary tangles in

- diffuse neurofibrillary tangles with calcification. *Acta Neuropathol*, Vol. 106, No. 2, pp. 150-156, ISSN 0001-6322
- Henkel, J.S., Beers, D.R., Zhao, W. & Appel, S.H. (2009). Microglia in ALS: the good, the bad, and the resting. *J Neuroimmune Pharmacol*, Vol. 4, No. 4, pp. 389-98, ISSN 1557-1904
- Herrera, A.J., Castano, A., Venero, J.L., Cano, J. & Machado, A. (2000). The single intranigral injection of LPS as a new model for studying the selective effects of inflammatory reactions on dopaminergic system. *Neurobiol Dis*, Vol. 7, No. 4, pp. 429-47, ISSN 0969-9961
- Hertz, L. (2008). Bioenergetics of cerebral ischemia: a cellular perspective. *Neuropharmacology*, Vol. 55, No. 3, pp. 289-309, ISSN 0028-3908
- Hirsch, E.C. & Hunot, S. (2009). Neuroinflammation in Parkinson's disease: a target for neuroprotection? *Lancet Neurol*, Vol. 8, No. 4, pp. 382-97, ISSN 1474-4422
- Katayama, Y., Maeda, T., Koshinaga, M., Kawamata, T. & Tsubokawa, T. (1995). Role of excitatory amino acid-mediated ionic fluxes in traumatic brain injury. *Brain Pathol*, Vol. 5, No. pp. 427-435, ISSN 1015-6305
- Kesslak, J., Yuan, D., Neepser, S. & Cotman, C. (1995). Vulnerability of the hippocampus to kainate excitotoxicity in the aged, mature and young adult rat. *Neurosci Letters*, Vol. 188, No. pp. 117-120, ISSN 0304-3940
- Kettenmann, H., Hanisch, U.K., Noda, M. & Verkhratsky, A. (2011). Physiology of microglia. *Physiol Rev*, Vol. 91, No. 2, pp. 461-553, ISSN 1522-1210
- Kim, K.M. (1995). Apoptosis and calcification. *Scanning Microscopy*, Vol. 9, No. 4, pp. 1137-1178, ISSN 0891-7035
- Kodaka, T., Mori, R., Debari, K. & Yamada, M. (1994). Scanning electron microscopy and electron probe microanalysis studies of human pineal concretions. *J Electron Microscopy*, Vol. 43, No. 5, pp. 307-317, ISSN 0022-0744
- Le Jeune, H., Cçcyre, D., Rowe, W., Meaney, M.J. & Quirion, R. (1996). Ionotropic glutamate receptor subtypes in the aged memory-impaired and unimpaired Long-Evans rat. *Neuroscience*, Vol. 74, No. 2, pp. 349-363, ISSN 0306-4522
- Li, C., Zhao, R., Gao, K., Wei, Z., Yin, M.Y., Lau, L.T., Chui, D. & Hoi Yu, A.C. (2011). Astrocytes: implications for neuroinflammatory pathogenesis of Alzheimer's disease. *Curr Alzheimer Res*, Vol. 8, No. 1, pp. 67-80, ISSN 1875-5828
- Liévens, J.C., Bernal, F., Forni, C., Mahy, N. & Kerkerian-Legoff, L. (2000). Characterization of striatal lesions produced by glutamate uptake alteration: cell death, reactive gliosis and changes in GLT1 and GADD45 mRNA expression. *Glia*, Vol. 29, No. pp. 222-232, ISSN 1098-1136
- Lull, M.E. & Block, M.L. (2010). Microglial activation and chronic neurodegeneration. *Neurotherapeutics*, Vol. 7, No. 4, pp. 354-65, ISSN 1878-7479
- Mahy, N., Diaz, R., Andrés, N. & Fuxe, K. (1996). Ibotenic acid in the medial septum increases brain-derived neurotrophic factor mRNA levels in the dorsal rat hippocampal formation. *Neurosci Letters*, Vol. 213, No. pp. 169-172, ISSN 0304-3940
- Mahy, N., Prats, A., Riveros, A., Andrés, N. & Bernal, F. (1999). Basal ganglia calcification induced by excitotoxicity: an experimental model characterised by electron microscopy and X-ray microanalysis. *Acta Neuropathol*, Vol. 98, No. 3, pp. 217-225, ISSN 0001-6322
- Mates, J.M., Perez-Gomez, C., Nunez De Castro, I., Asenjo, M. & Marquez, J. (2002). Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death. *Int J Biochem Cell Biol*, Vol. 34, No. 5, pp. 439-58, ISSN 1357-2725

- Mattson, M.K. & Chan, S.L. (2003). Neuronal and glial calcium signalling in Alzheimer's disease. *Cell Calcium*, Vol. 34 No. pp. 385-397, ISSN 0143-4160
- Mattson, M.P. (2006). Neuronal life-and-death signaling, apoptosis, and neurodegenerative disorders. *Antioxid Redox Signal.*, Vol. 8, No. 11-12, pp. 1997-2006, ISSN 1523-0864
- Milligan, E.D. & Watkins, L.R. (2009). Pathological and protective roles of glia in chronic pain. *Nat Rev Neurosci*, Vol. 10, No. 1, pp. 23-36, ISSN 1471-0048
- Mullany, P., Connolly, S. & Lynch, M.A. (1996). Ageing is associated with changes in glutamate release, protein tyrosine kinase and Ca²⁺/calmodulin-dependent protein kinase II in rat hippocampus. *Eur J Pharmacol*, Vol. 309, No. pp. 311-315, ISSN 0014-2999
- Nicolle, M.M., Bizon, J.L. & Gallagher, M. (1996). In vitro autoradiography of ionotropic glutamate receptors in hippocampus and striatum of aged Long-Evans rats: relationship to spatial learning. *Neuroscience*, Vol. 74, No. 3, pp. 741-756, ISSN 0306-4522
- Obrenovitch, T.P., Urenjak, J., Zilkha, E. & Jay, T.M. (2000). Excitotoxicity in neurological disorders--the glutamate paradox. *Int J Dev Neurosci*, Vol. 18, No. 2-3, pp. 281-287, ISSN 0736-5748
- Olney, J.W., Wozniak, D.F. & Farber, N.B. (1997). Excitotoxic neurodegeneration in Alzheimer disease. *Arch Neurol*, Vol. 54, No. pp. 1234-1240, ISSN 0003-9942
- Ovanesov, M.V., Ayhan, Y., Wolbert, C., Moldovan, K., Sauder, C. & Pletnikov, M.V. (2008). Astrocytes play a key role in activation of microglia by persistent Borna disease virus infection. *J Neuroinflammation*, Vol. 5, No. pp. 50, ISSN 1742-2094
- Pellerin, L., Bouzier-Sore, A.K., Aubert, A., Serres, S., Merle, M., Costalat, R. & Magistretti, P.J. (2007). Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia*, Vol. 55, No. 12, pp. 1251-1262, ISSN 1098-1136
- Perry, V.H., Nicoll, J.A. & Holmes, C. (2010). Microglia in neurodegenerative disease. *Nat Rev Neurol*, Vol. 6, No. 4, pp. 193-201, ISSN 1759-4766
- Petegnief, V., Saura, J., Dewar, D., Cummins, D.J., Dragunow, M. & Mahy, N. (1999). Long-term effects of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate and 6-nitro-7-sulphamoylbenzo(f)quinoxaline-2,3-dione in the rat basal ganglia: calcification, changes in glutamate receptors and glial reactions. *Neuroscience*, Vol. 94, No. 1, pp. 105-115, ISSN 0306-4522
- Petegnief, V., Ursu, G., Bernal, F. & Mahy, N. (2004). Nimodipine and TMB-8 potentiate the AMPA-induced lesion in the basal ganglia. *Neurochem Int*, Vol. 44 No. pp. 287-291, ISSN 0197-0186
- Philips, T. & Robberecht, W. (2011). Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. *Lancet Neurol*, Vol. 10, No. 3, pp. 253-63, ISSN 1474-4465
- Ramonet, D., De Yebra, L., Fredriksson, K., Bernal, F., Ribalta, T. & Mahy, N. (2006). Similar calcification process in acute and chronic human brain pathologies. *J Neurosci Res*, Vol. 83 No. pp. 147-156, ISSN 0360-4012
- Ramonet, D., Pugliese, M., Rodríguez, M.J., De Yebra, L., Andrade, C., Adroer, R., Ribalta, T., Mascort, J. & Mahy, N. (2002). Calcium precipitation in acute and chronic brain diseases. *J Physiol-Paris*, Vol. 96, No. pp. 307-312, ISSN 0928-4257
- Ramonet, D., Rodríguez, M.J., Fredriksson, K., Bernal, F. & Mahy, N. (2004). In vivo neuroprotective adaptation of the glutamate/glutamine cycle to neuronal death. *Hippocampus*, Vol. 14, No. 5, pp. 586-594, ISSN 1098-1063

- Ransohoff, R.M. & Perry, V.H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu Rev Immunol*, Vol. 27, No. pp. 119-45, ISSN 0732-0582
- Reali, C., Scintu, F., Pillai, R., Donato, R., Michetti, F. & Sogos, V. (2005). S100b counteracts effects of the neurotoxicant trimethyltin on astrocytes and microglia. *J Neurosci Res*, Vol. 81, No. 5, pp. 677-86, ISSN 0360-4012
- Rivers, L.E., Young, K.M., Rizzi, M., Jamen, F., Psachoulia, K., Wade, A., Kessar, N. & Richardson, W.D. (2008). PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. *Nat Neurosci*, Vol. 11, No. 12, pp. 1392-401, ISSN 1546-1726
- Robledo, P., Ursu, G. & Mahy, N. (1999). Effects of adenosine and gamma-aminobutyric acid A receptor antagonists on N-methyl-D-aspartate induced neurotoxicity in the rat hippocampus. *Hippocampus*, Vol. 9, No. 5, pp. 527-533, ISSN 1098-1063
- Rodriguez, M.J., Bernal, F., Andres, N., Malpesa, Y. & Mahy, N. (2000). Excitatory amino acids and neurodegeneration: a hypothetical role of calcium precipitation. *Int J Dev Neurosci*, Vol. 18, No. 2-3, pp. 299-307, ISSN 0736-5748
- Rodriguez, M.J., Martinez-Sanchez, M., Bernal, F. & Mahy, N. (2004). Heterogeneity between hippocampal and septal astroglia as a contributing factor to differential in vivo AMPA excitotoxicity. *J Neurosci Res*, Vol. 77, No. 3, pp. 344-353, ISSN 0360-4012
- Rodriguez, M.J., Prats, A., Malpesa, Y., Andres, N., Pugliese, M., Batlle, M. & Mahy, N. (2009a). Pattern of injury with a graded excitotoxic insult and ensuing chronic medial septal damage in the rat brain. *J Neurotrauma*, Vol. 26, No. 10, pp. 1823-34, ISSN 1557-9042
- Rodriguez, M.J., Pugliese, M. & Mahy, N. (2009b). Drug abuse, brain calcification and glutamate-induced neurodegeneration. *Curr Drug Abuse Rev*, Vol. 2, No. 1, pp. 99-112, ISSN 1874-4745
- Rodriguez, M.J., Robledo, P., Andrade, C. & Mahy, N. (2005). In vivo co-ordinated interactions between inhibitory systems to control glutamate-mediated hippocampal excitability. *J Neurochem*, Vol. 95, No. 3, pp. 651-661, ISSN 0022-3042
- Rodriguez, M.J., Ursu, G., Bernal, F., Cusi, V. & Mahy, N. (2001). Perinatal human hypoxia-ischemia vulnerability correlates with brain calcification. *Neurobiol Dis*, Vol. 8, No. pp. 59-68, ISSN 0969-9961
- Safadi, M.A., Berezin, E.N., Farhat, C.K. & Carvalho, E.S. (2003). Clinical presentation and follow up of children with congenital toxoplasmosis in Brazil. *Braz J Infect Dis*, Vol. 7, No. 5, pp. 325-31, ISSN 1413-8670
- Sapolsky, R.M. (2001). Cellular defenses against excitotoxic insults. *J Neurochem*, Vol. 76, No. pp. 1601-1611, ISSN 0022-3042
- Saura, J., Andrés, N., Andrade, C., Ojuel, J., Eriksson, K. & Mahy, N. (1997). Biphasic and region-specific MAO-B response to aging in normal human brain. *Neurobiol Aging*, Vol. 18, No. 5, pp. 497-507, ISSN 0197-4580
- Saura, J., Boatell, M.L., Bendahan, G. & Mahy, N. (1995). Calcium deposit formation and glial reaction in rat brain after ibotenic acid-induced basal forebrain lesion. *Eur J Neurosci*, Vol. 7, No. 7, pp. 1569-1578, ISSN 0953-816X
- Saura, J., Richards, J. & Mahy, N. (1994). Age-related changes of MAO in BL/C57 mouse tissues: A quantitative radioautographic study. *J Neural Transm*, Vol. 41, No. pp. 89-94, ISSN 1435-1463
- Schinder, A.F., Olson, E.C., Spitzer, N.C. & Montal, M. (1996). Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. *J Neurosci*, Vol. 16, No. 19, pp. 6125-6133, ISSN 0270-6474

- Schwartz, M., Butovsky, O., Bräck, W. & Hanisch, U.K. (2006). Microglial phenotype: is the commitment reversible? *Trends Neurosci*, Vol. 29 No. 2, pp. 68-74, ISSN 0166-2236
- Seifert, G., Schilling, K. & Steinhilber, C. (2006). Astrocyte dysfunction in neurological disorders: a molecular perspective. *Nat Rev Neurosci*, Vol. 7, No. pp. 194-206, ISSN 1471-003X
- Sibson, N.R., Dhankhar, A., Mason, G.F., Rothman, D.L., Behar, K.L. & Shulman, R.G. (1998). Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proc Natl Acad Sci USA*, Vol. 95, No. pp. 316-321, ISSN 0027-8424
- Sofroniew, M.V. & Vinters, H.V. (2010). Astrocytes: biology and pathology. *Acta Neuropathol*, Vol. 119, No. 1, pp. 7-35, ISSN 1432-0533
- Stoll, G., Jander, S. & Schroeter, M. (2002). Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. *Adv Exp Med Biol*, Vol. 513, No. pp. 87-113, ISSN 0065-2598
- Stout, A.K., Raphael, H.M., Kanterewicz, B.L., Klann, E. & Reynolds, I.J. (1998). Glutamate-induced neuron death requires mitochondrial calcium uptake. *Nat Neurosci*, Vol. 1, No. 5, pp. 366-373, ISSN 1097-6256
- Streit, W.J., Mark, R. & Griffin, S. (2004). Microglia and neuroinflammation: a pathological perspective. *J Neuroinflammation*, Vol. 1, No. pp. 14-17, ISSN 1742-2094
- Surendrababu, N.R., Kuruvilla, K.A., Jana, A.K. & Cherian, R. (2006). Globe calcification in congenital toxoplasmosis. *Indian J Pediatr*, Vol. 73, No. 6, pp. 527-8, ISSN 0973-7693
- Suzumura, A., Takeuchi, H., Zhang, G., Kuno, R. & Mizuno, T. (2006). Roles of glia-derived cytokines on neuronal degeneration and regeneration. *Ann N Y Acad Sci*, Vol. 1088, No. pp. 219-29, ISSN 0077-8923
- Tanuma, N., Sakuma, H., Sasaki, A. & Matsumoto, Y. (2006). Chemokine expression by astrocytes plays a role in microglia/macrophage activation and subsequent neurodegeneration in secondary progressive multiple sclerosis. *Acta Neuropathol*, Vol. 112, No. 2, pp. 195-204, ISSN 0001-6322
- Tramontina, F., Tramontina, A.C., Souza, D.F., Leite, M.C., Gottfried, C., Souza, D.O., Wofchuk, S.T. & Goncalves, C.A. (2006). Glutamate uptake is stimulated by extracellular S100B in hippocampal astrocytes. *Cell Mol Neurobiol*, Vol. 26, No. 1, pp. 81-6, ISSN 0272-4340
- Van Eldik, L.J. & Wainwright, M.S. (2003). The Janus face of glial-derived S100B: beneficial and detrimental functions in the brain. *Restor Neurol Neurosci*, Vol. 21, No. 3-4, pp. 97-108, ISSN 0922-6028
- Verkhatsky, A.J. (2007). Calcium and cell death. *Subcell Biochem.*, Vol. 45, No. pp. 465-480, ISSN 0096-8757
- Verkhatsky, A.J. (2005). Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. *Physiol Rev*, Vol. 85 No. pp. 201-279, ISSN 0031-9333
- Villa, A., Podini, P., Panzeri, M.C., Racchetti, G. & Meldolesi, J. (1994). Cytosolic Ca²⁺ binding proteins during rat brain ageing: loss of calbindin and calretinin in the hippocampus, with no change in the cerebellum. *Eur J Neurosci*, Vol. 6, No. pp. 1491-1499, ISSN 0953-816X
- Wake, H., Moorhouse, A.J., Jinno, S., Kohsaka, S. & Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci*, Vol. 29, No. 13, pp. 3974-80, ISSN 1529-2401



Neurodegenerative Diseases - Processes, Prevention, Protection and Monitoring

Edited by Dr Raymond Chuen-Chung Chang

ISBN 978-953-307-485-6

Hard cover, 558 pages

Publisher InTech

Published online 09, December, 2011

Published in print edition December, 2011

Neurodegenerative Diseases - Processes, Prevention, Protection and Monitoring focuses on biological mechanisms, prevention, neuroprotection and even monitoring of disease progression. This book emphasizes the general biological processes of neurodegeneration in different neurodegenerative diseases. Although the primary etiology for different neurodegenerative diseases is different, there is a high level of similarity in the disease processes. The first three sections introduce how toxic proteins, intracellular calcium and oxidative stress affect different biological signaling pathways or molecular machineries to inform neurons to undergo degeneration. A section discusses how neighboring glial cells modulate or promote neurodegeneration. In the next section an evaluation is given of how hormonal and metabolic control modulate disease progression, which is followed by a section exploring some preventive methods using natural products and new pharmacological targets. We also explore how medical devices facilitate patient monitoring. This book is suitable for different readers: college students can use it as a textbook; researchers in academic institutions and pharmaceutical companies can take it as updated research information; health care professionals can take it as a reference book, even patients' families, relatives and friends can take it as a good basis to understand neurodegenerative diseases.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jose M. Vidal-Taboada, Nicole Mahy and Manuel J. Rodríguez (2011). Microglia, Calcification and Neurodegenerative Diseases, Neurodegenerative Diseases - Processes, Prevention, Protection and Monitoring, Dr Raymond Chuen-Chung Chang (Ed.), ISBN: 978-953-307-485-6, InTech, Available from: <http://www.intechopen.com/books/neurodegenerative-diseases-processes-prevention-protection-and-monitoring/microglia-calcification-and-neurodegenerative-diseases>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

