

Hyperfunction (Alzheimer's disease and Parkinson disease) and hypofunction (schizophrenia) of glutamatergic neurons

Magnesium deficiency in Alzheimer's disease and Parkinson disease (as a loss of parasympathetic function?); calcium deficiency in schizophrenia?

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Introduction

Neurodegeneration is a multifaceted process involving, among others, the unbalance of the glutamatergic system. Prolonged exposure of neurons to moderate-to-high concentrations of L-glutamate irreversibly culminates in neuronal damage. It is generally accepted that the influx of Ca^{2+} as a result of **excessive activation of the NMDA** receptor underlies the toxic actions of glutamate in many systems. Also, acute ammonia intoxication leads to **excessive activation of NMDA** receptors in brain (HERMENEGILDO et al., 2000), which is responsible for ammonia-induced death of animals (MARCAIDA et al., 1992; HERMENEGILDO et al., 1996). Because of their central role in neurodegeneration, NMDA receptors have been considered prime therapeutic targets for the development of useful neuroprotective strategies (BRAUNER-OSBORNE et al., 2000). Accordingly, a significant effort has been made to develop high-affinity and selective NMDA antagonists that target the different drug binding sites on this receptor. Although most of these molecules efficiently reduce glutamate neurotoxicity in vitro, their in vivo utility has been heavily questioned **due to serious side effects at clinically effective doses** (CHOI and ROTHMAN, 1990; MORRIS and DAVIS, 1995; BRAUNER-OSBORNE et al., 2000). The high receptor affinity of known NMDA receptor antagonists appears to be a major shortcoming because these **compounds bind to both pathologically activated and physiologically working NMDA receptor populations** (CHOI and ROTHMAN, 1990; KROEMER et al., 1998).

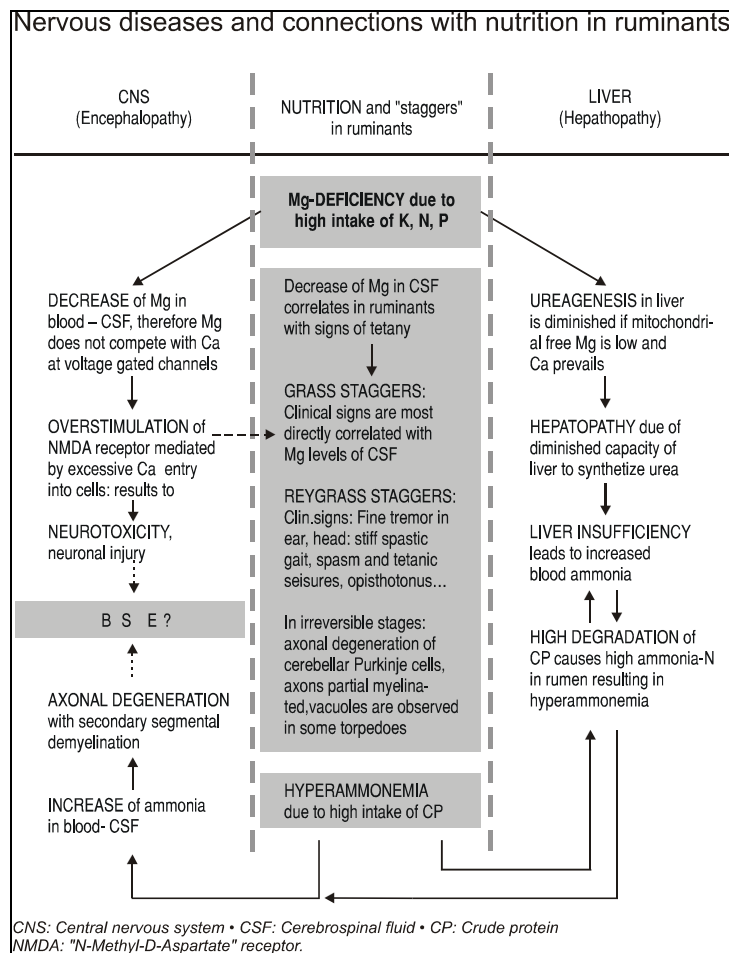
Glutamate is considered the main excitatory neurotransmitter in mammals. However, excessive activation of glutamate receptors, particularly of the NMDA receptor subtype, leads to neuronal degeneration and death (CHOI and ROTHMAN, 1990). **Glutamate neurotoxicity** is involved in the neuronal damage found in cerebral ischemia, as well as in the **pathogenesis of different neurodegenerative diseases**. Although the underlying mechanisms for the selective vulnerability of neurons are unknown, a widely held view considers that excessive activation of NMDA receptor mediates a **massive influx of Ca^{2+} , which induces different**

effects, including alterations of the mitochondrial potential and formation of nitric oxide and cGMP. These alterations lead ultimately to cell death (CHOI, 1987; DAWSON et al., 1991; DAWSON et al., 1993; MATTSON et al., 1993; MONTOLIU et al., 1999). However, overstimulation of the NMDA receptor by glutamate is also implicated in Alzheimer's disease. Accordingly, REISBERG et al. (2003) investigated memantine, an NMDA antagonist, for the treatment of Alzheimer's disease. **Antiglutamatergic treatment reduced clinical deterioration** in moderate-to-severe Alzheimer's disease, a phase associated with distress for patients and burden on caregivers, for which other treatments are not available.

Since NMDA receptors (NMDAR) are one of the most harmful factors in **excitotoxicity**, antagonists of the receptors have held much promise for the treatment of conditions that involve excitotoxicity, including traumatic brain injury, stroke, and **neurodegenerative diseases**. **Energetically compromised neurons become depolarized** (more positively charged) because in the absence of energy they cannot maintain ionic homeostasis; this depolarization relieves the normal Mg^{2+} block of NMDA receptor-coupled channels because the relatively positive charge in the cell repels positively-charged Mg^{2+} from the channel pore. Hence, **during periods of ischemia and in many neurodegenerative diseases, excessive stimulation of glutamate receptors is thought to occur**. These neurodegenerative diseases, including **Alzheimer's disease, Parkinson's disease, Huntington's disease...**, are caused by different mechanisms but may share a final **common pathway to neuronal injury due to the overstimulation of glutamate receptors**, especially of the NMDA subtype (LIPTON and ROSENBERG, 1994).

And what is known about **overstimulation NMDA receptor** in other **neurodegenerative** „mad cow disease” (BSE)? I described an alternative „ammonia- magnesium“ BSE theory (March, 2001)- see the Bulletin of Research Institute of Cattle Breeding in Rapotín , Czech Republic (see Fig 1) see also this text reprinted in international journal „Feed-Mix“(2002) (<http://www.agriworld.nl/feedmix/headlines.asp?issue=3>).

Fig. 1



Concerning the association between the autonomic nervous system function and the BSE incidence; much evidence suggests that so-called prions are harmless, noninfectious products. The importance of the cholinergic system allows a new simplified interpretation of these conditions. According to AXELSSON (2001), a change in handedness (chirality) in some acids appears to be the basic physical change in degradation-resistant proteins (prions) found in conditions such as CJD, Alzheimer's disease, BSE, and ovine scrapie. The affected structures are primarily innervated by cholinergic nerves. The main steps are the acetylcholine- cholinesterase splitting of body water with release of free protons in solution, followed by electron dissipation, dioxygen activation and Ca- fluxes (AXELSSON, 2001)

On the other hand a hypofunction of glutamatergic neurons has been hypothesized to caused schizophrenia. Non-competitive NMDA antagonists, in addition to their neuroprotective potential, possess neurotoxic properties and induce seizures and psychosis. MK-801 induces cytoplasmic vacuoles and heat shock protein in pyramidal neurones in the rodent posterior cingulate and retrosplenial cortex. The mechanism of this neurotoxicity is unclear, involving many neurotransmitter systems. The aim of the study (WILLIS et al., 2006) was to investigate the role of cholinergic pathways from the nucleus basalis of Meynert in mediating MK-801-induced neurotoxicity.. These results demonstrate that cholinergic neurones in the nucleus basalis of Meynert play an important role in the heat shock response to NMDA antagonist-induced neurotoxicity but also reveal an unexpected divergence between the heat shock response and the pathophysiological response. This suggests that other cholinergic pathways or non-cholinergic mechanisms are responsible for the pathological changes induced by MK-801 (WILLIS et al., 2006).

Findings of reduced concentrations of glutamate in the cerebrospinal fluid of patients with schizophrenia and the ability of glutamate-receptor antagonists to cause psychotic symptoms lend support to this hypothesis (TSAI et al., 1995). They also suggest that the therapeutic efficacy of neuroleptics may be related to increased glutamatergic activity. Evidence from histological and pharmacological challenge studies indicates that NMDA receptor hypofunction may play an important role in the pathophysiology of schizophrenia. It has long been known that treatment with NMDA receptor antagonists produces psychosis and cognitive deficits that are reminiscent of the clinical picture of schizophrenia (JAVITT and ZUKIN, 1991; KRYSTAL et al., 1994).

These data led to the NMDA receptor **hypofunction model of schizophrenia (OLNEY and FARBER, 1994; 1995)**. Authors have been studying two parallel phenomena: NMDA-antagonist neurotoxicity (NAN) in rats and NMDA-antagonist psychotogenicity (NAP) in humans. These phenomena have a common denominator--NMDA receptor hypofunction, which is putatively a mechanism operative in schizophrenia. They have found that the NAN reaction in rats can be prevented by specific drugs that prevent NAP in humans and by certain antipsychotic agents, including clozapine, that ameliorate symptoms in schizophrenia. By studying mechanisms by which clozapine prevents the NAN reaction in rats, they hope to gain insight into mechanisms by which clozapine or other atypical antipsychotics ameliorate symptoms in schizophrenia (OLNEY and FARBER, 1994). They begin by explaining the concept of NMDA receptor hypofunction (NRH) and its history, and present evidence from research that supports NRH and its links to various aspects of schizophrenia. According to OLNEY and FARBER, the NRH hypothesis, or **glutamate hypothesis, was capable of explaining: the positive and negative symptoms of schizophrenia, the early onset of the illness, structural brain abnormalities, response to neuroleptic treatment, and cognitive deterioration.**

The hypothesis that NMDA receptor hypofunction might be important in schizophrenia was not given much credence until investigators performed experiments in which **healthy volunteers received subanesthetic infusions of the dissociative anesthetic ketamine, a known NMDA receptor antagonist**. The infusions reproduced the positive and negative symptoms of schizophrenia, including paranoia, thought disorder, loose associations, illusions, emotional withdrawal, and psychomotor retardation. **NMDA receptor antagonists** have been investigated for many years as therapeutic agents for the treatment of neurological disorders such as stroke, epilepsy, pain and Parkinson's disease.

The goal of RADANT et al.(1998) was to characterize effects of NMDA hypofunction further, as related to schizophrenia-associated neuropsychological impairment. They **administered progressively higher doses of ketamine to 10 psychiatrically healthy** young men. They concluded that ketamine induces changes in recall and recognition memory and verbal fluency reminiscent of schizophreniform psychosis. In a symposium entitled, "**Not Just Dopamine Any More: Emerging Glutamatergic Therapies for Schizophrenia,**" (2006) professor Joseph Coyle from Harvard Medical School, Cambridge, Massachusetts, and Editor of the Archives of General Psychiatry, described molecular mechanisms that had recently been identified as being of interest in schizophrenia .

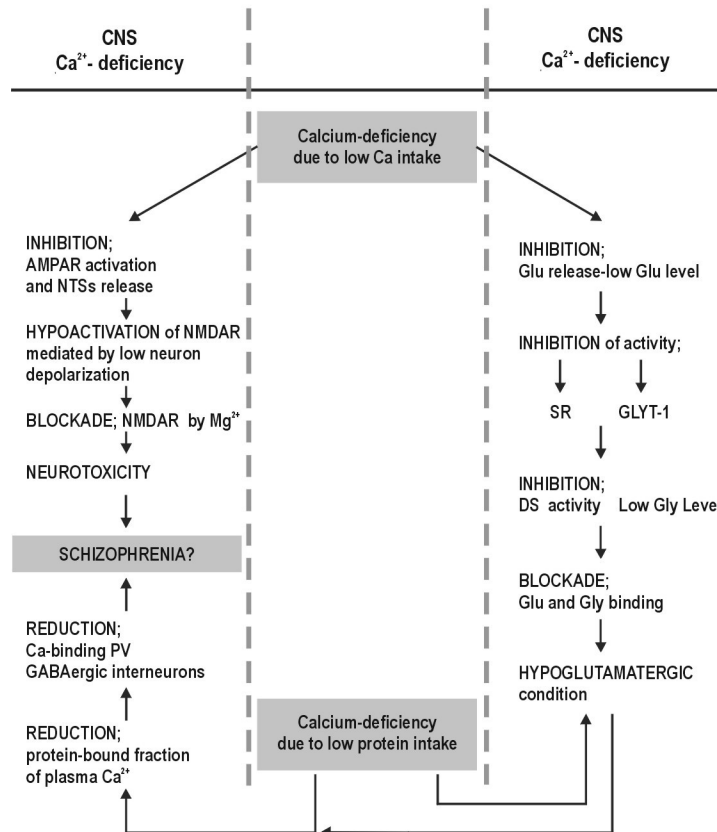
However, treatment studies with NMDA modulators, such as glycine, D-serine, and glycine transport inhibitors (GTIs), have yielded encouraging findings, although results remain controversial. Why? Because- perhaps, NMDA receptors might have a lower affinity for glycine, explaining why administration of exogenous glycine-agonists results in **a favorable clinical response in schizophrenia**. Additionally, one could imagine that these **receptors might be less sensitive to glutamate**, and, perhaps, more sensitive to Mg^{2+} block . So, **NMDA receptors may differ in their sensitivity to voltage-dependent Mg^{2+} block**, agonists, and antagonists as a function of their subunit composition.

The activity of NMDA receptors is strikingly sensitive to the changes in H^+ concentration, and partially inhibited by the ambient concentration of H^+ under physiological conditions. The frequency of NMDA receptor channel openings is **reduced by protons over the physiological pH range**, with a midpoint at pH 7.4, such that at pH 6.0 receptor activation is suppressed nearly completely (NOWAK et al., 1984). There can be other explanation from professor BEČKA about **treatment studies and „controversial results“**. He concluded (1929-1935), that the tonicity of the **parasympathetic nervous system** is maintained (long term „enterally“) by Mg^{2+} and OH^- ; and that of the **sympathetic system by Ca^{2+} and H^+** (homeopathic doses). However (short-term „parenterally“),overdosage of Ca^{2+} (and H^+) causes the inhibition of the sympathetic and the parasympathetic nervous system action prevails. Similarly, overdosage of the Mg^{2+} (and OH^-) causes the inhibition of the parasympathetic and the sympathetic nervous system prevails. So, he considered that the actual control is a negative feedback mechanism, and, importantly, professor Becka discovered that this **mechanism is influenced by the dosage of Ca^{2+} and Mg^{2+} in connection with the acid-base state of animals** (HLÁSNÝ,2000). However, diverse magnesium salts (enteral or parenteral), may have different effects about calcium losses by faeces, urine and influence on acid-base status; which differs **according to the nature of the anions** (BEČKA, 1936; 1936a).

Functional diversity of NMDA receptors may be expected from the assembly of different subunit combinations, and there is very **important „ Ca^{2+} -dependent manner” which permits activation of NMDA receptors**. Calcium metabolism or calcium homeostasis is the mechanism by which the body maintains adequate calcium levels. However, **derangements of this mechanism can lead to “calcium- deficiency”,** which can have important

consequences in health of “schizophrenic individuals”. This concept is based on the demonstration that „NMDA receptor hypofunction“ can be based on calcium-deficiency, potentiated by nutritional hypoproteinemia (Fig.2);

Fig.2 Calcium deficiency may be a predisposing or causative factor in schizophrenia



CNS (Central nervous system) • NMDAR (N-Methyl-D-Aspartate receptor) • AMPAR (Amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor) • NTSs (Neurotransmitters) • Glu (Glutamate) • Gly (Glycine) • GLYT-1 (Glycine transporter type 1) • DS (D-serine) • SR (Serine racemase enzyme) • PV (Parvalbumin) • GABA (Gamma- amino-butyric acid)

So, dietary calcium deficiency can be important about „NMDA hypofunction“ in schizophrenia. However, there can be another example about hypoglutamatergic condition; **cannabinoids are known to inhibit Ca²⁺ channels- glutamate release in schizophrenia.** Some studies suggest that cannabis is neither a sufficient nor necessary factor in developing schizophrenia, but that cannabis may significantly increase the risk of developing schizophrenia and may be, among other things, a significant causal factor. Nevertheless, some previous research in this area has been criticised as it has often not been clear whether cannabis use is a **cause or effect of schizophrenia.** The goal of this view is to show that cannabis use can be a cause of schizophrenia; characterize two effects on NMDA hypofunction, related to schizophrenia-associated neurodegenerative impairment;

1. Cannabinoids activity about NMDA glutamate receptor hypofunction; as a schizophreniform effect;

In expression systems and cell bodies, CB1 receptor couples to activation of K⁺ channels or inhibition of neuronal Ca²⁺ channels, or both. Either of these mechanisms can reduce Ca²⁺

influx at nerve terminals and thereby inhibit transmitter release. Activation of K^+ channels may change the presynaptic action potential and thus indirectly modulate Ca^{2+} channel activity. So, communication between the cells requires the release of a glutamate neurotransmitter, triggered by calcium currents passing through a specific Ca^{2+} channel. Cannabinoids are known to inhibit Ca^{2+} channels. If we shut down the channel, we shut down the release of glutamate, and profoundly alter the cell's ability to signal.

2. Cannabinoids can regulate NMDA glutamate receptor by reducing intracellular Ca^{2+} release ; as a neuroprotective effect;

There is evidence that cannabinoids can regulate glutamate release, oxidant free radicals and calcium influxes, which, in excess, can cause neuronal death. Cannabinoids can tonically regulate NMDA glutamate receptor activity in vitro and support the in vivo observation that CB_1 regulates NMDA-induced and ischaemic excitotoxicity. Exogenously administered cannabinoids are neuroprotective in several different cellular and animal models. Cannabinoids produce neuroprotection by reducing intracellular Ca^{2+} release. Emerging evidence indicates that cannabinoids may play a role in slowing the progression of certain neurodegenerative diseases, such as Multiple Sclerosis, Parkinson's disease, Alzheimer's, and Amyotrophic Lateral Sclerosis (ALS).

Alzheimer's disease

Meat and dairy-based diets contribute to Alzheimer's disease, while low protein-based diets protected from it

What causes Alzheimer's disease (AD)?

Scientists have identified several factors that appear to play in the development of AD but have not yet reached (to date) any firm conclusions as to exactly what causes the disease. There are some theories, e.g.; that high blood pressure may damage blood vessels in brain. That oxidative damage refers to cell damage caused by excess free radicals it may cause substantial neuronal damage, contributing to AD development... (excitotoxicity has been implicated in the etiology of ischemic stroke and chronic neurodegenerative disorders).

Improperly formed proteins are linked to a number of diseases, including Parkinson's, Alzheimer's, cystic fibrosis, amyotrophic lateral sclerosis, also known as Lou Gherig's disease, and bovine spongiform encephalopathy, better known as mad-cow disease. There are a number of neurodegenerative diseases, but, other than **Alzheimer's disease, which affects 15,000 people per million population**, most are unfamiliar and rare. Creutzfeldt-Jakob Disease (CJD) is the rarest of all, affecting only one person per million population. Other neurodegenerative diseases include **Parkinson's disease (3,600 cases per million population)**; Frontotemporal dementia (140/million); Huntingdon's disease (folk singer Woody Guthrie is the most famous victim of this disease which occurs at a rate of 110/million); Amyotrophic lateral sclerosis (also called Lou Gehrig's disease, has an incidence of 70/million); Progressive supranuclear palsy (50/million); Spinocerebellar ataxias (40/million); and Pick's disease (20/million).

More than 4.5 million Americans are believed to have Alzheimer's disease and by 2050, the number could increase to 13.2 million. Approximately 59,000 victims die and 350,000

new cases of Alzheimer's disease are diagnosed each year. America is not alone in dealing with this terrible affliction. In every nation where life expectancy has increased, so has the incidence of Alzheimer's disease. Alzheimer's disease is becoming tragically common. It is estimated that there are currently 18 million people worldwide with Alzheimer's disease. This figure is projected to nearly double by 2025 to 34 million people.

We have known for quite a long time that **Alzheimer's disease (AD) is associated with a loss of cholinergic neurons** resulting in profound memory disturbances and irreversible impairment of cognitive function. Also is well known that Alzheimer's Disease was associated with buildups of neuritic plaque- globs and fibers of hard, insoluble material in the brain's neurons. For most of that time, it wasn't known if these plaques were the cause of Alzheimer's, or a secondary effect of whatever was causing the primary damage. It turns out that amyloid precursor protein is broken down by the body into a very small (40 amino acids long, actually) protein called Ab. This protein is usually soluble, but with Alzheimer's the Ab proteins begin to accumulate together in insoluble amounts. The harmless Ab becomes the disease-causing plaque. Diseases caused by prions, like **Mad Cow / Creutzfeldt-Jacob** are also, in essence, protein folding disorders. These are caused by a certain protein, named PrP, that will stay in a mis-folded conformation (PrPsc) if encouraged to go into it in the first place. While PrP can be processed and cleaned out of a cell once it has been used, PrPsc is shaped differently enough that it can't be, so it never goes away. PrPsc, much more quickly than with Ab in Alzheimer's, builds up into plaques, handily destroying whatever nervous tissue it's building up in.

Neuronal vacuolation (spongiosis), neuronal death, and pronounced glial reactions are the hallmarks of transmissible spongiform encephalopathies (TSEs), or prion diseases. A wealth of physical, biochemical, and immunological evidence indicates that the TSE agent, termed prion, does not contain agent-specific nucleic acid encoding its own constituents, as is the case for all other infectious pathogens. Also, no adaptive immune responses are elicited upon infection. A defining feature of TSEs is the deposition, mainly in the brain and lymphoreticular tissues, of an aggregated and structurally abnormal protein, designated PrP(Sc) or PrP-res, which represents a conformational isomer of the ubiquitous surface protein PrP(C). Biochemical and genetic evidence link PrP and its gene to the disease. **Although TSEs are by definition transmissible, a growing number of Prnp-associated non-infectious neurodegenerative proteinopathies are now being recognized** (AGUZZI, 2006).

The findings suggest that the malformed protein clumps associated with Alzheimer's disease can "seed" themselves in a way reminiscent of the misfolded proteins in prion diseases such as "mad cow" disease. The exact causes of Alzheimer's remain a mystery, but it appears that beta-amyloid proteins contribute to the formation of disruptive plaques in the brain. The neurological damage accumulates over years, causing loss of memory, language and other crucial mental skills. Experts studying how beta amyloid might promote plaque formation have speculated that this might happen in a process similar to that in prion diseases.

Link between eating processed meat (high protein intake) and Alzheimer's disease?

The link between eating processed meat and Alzheimer's disease unfolds as a remarkable narrative, one of the most fascinating stories in modern medicine. According to a highly controversial book published in september 2004, prions could also be the cause of Alzheimer's disease. The book, already published in Canada and criticised by both the meat-

packing industry and the Alzheimer's establishment, was entitled „*Dying for a Hamburger: how modern meat-packing led to an epidemic of Alzheimer's disease*“. Co-written by Dr Murray Waldman, a Canadian coroner and researcher at the University of Toronto, it raises a number of worrying questions about an incurable condition that currently threatens 20 per cent of people over 80.

Waldman begins by asking: “Is Alzheimer's a new disease?” Although its cause is unknown, most accounts tend to assume that cognitive decline has always been associated with growing old. However, Waldman's review of pre-20th-century literature shows no evidence for our assumption that old age goes with mental decline. In fact, the medical definition of “senile” did not include “dementia” until 1962. Most remarkably, the number of articles on Alzheimer's disease in medical journals increased by 5,000 times between 1966 and 2000, compared with six times and four times for prostate cancer and heart disease. “It is hard to avoid the conclusion that Alzheimer's is a new disease,” Waldman writes.

In his book Dying for a Hamburger, Dr. Murray Waldman makes the case that the connection between meat consumption and Alzheimer's disease may result from certain proteins called prions, which contribute to the onset of the disease. Similar to the infectious agent that causes mad cow disease and the human version, Creutzfeldt-Jakob's disease, these prions are misshapen versions of proteins that are normally found in the nervous systems of animals. When prions come into contact with normal, functioning proteins, they cause these proteins to become misshapen, too. As the chain reaction continues, more and more proteins become misshapen and begin to impair normal neurological functions.

Waldman demonstrates that Alzheimer's disease first showed up in medical records at about the same time that world meat consumption began to rise. There is a direct correlation between the rates of meat consumption and the rates of Alzheimer's disease in various cultures across the world. In Africa and China and other Asian countries, where meat consumption is relatively low, the rates of Alzheimer's disease are much lower than in the United States, Canada, the United Kingdom, and other developed countries, where meat consumption is high.

The villain, he claims, is some sort of prion, an abnormal protein found in brain tissue which clumps together with other proteins and is responsible for certain rare neurological disorders known as “prion diseases”. To begin with there is guilt by association. The **similarities between Alzheimer's and prion diseases- BSE/ vCJD** are not the only ones-include taking a long time to emerge, driving their victims mad and being associated with plaques and tangles in the brain. What's more, **cases of vCJD can be misdiagnosed as Alzheimer's and vice versa**. Crucially, there is the fact that Alzheimer's emerged in the wake of the industrialised farming and meat-packaging production-line techniques that allowed the BSE prions to get into the food chain. Where these techniques have been introduced, first in Europe and America and more recently in the countries of the Pacific Rim, processed meat consumption has soared along with rates of Alzheimer's. Waldman points out that **levels of Alzheimer's in largely vegetarian India are still very low**. He makes a fascinating case based on circumstantial evidence, but it lacks a smoking gun. The clincher would be if it were possible to inject tissue from the brains of Alzheimer's victims into animals and recreate the disease in them, as can be done with vCJD. Research of this kind has not been undertaken in the UK but experts here are adamant that it cannot be done, and that Alzheimer's is not a prion disease. “Both diseases involve proteins in the brain that somehow change shape and cause damage,” says Dr Susanne Sorensen, head of research for the Alzheimer's Society. “But they are different proteins and the diseases have a different biochemistry and a different pathology.” Her

explanation for the link with raised meat consumption is that this is a marker for the Western high-fat diet that is also blamed for heart disease. “Many of the risk factors for heart disease also apply to Alzheimer’s,” she says.

Link between diet rich in fruits, vegetables, fish, legumes, cereals and olive oil (low protein intake) and Alzheimer’s disease?

Americans who ate a Mediterranean diet--lots of fruits, vegetables, legumes, cereals, some fish and alcohol, and little dairy and meat--had a reduced risk for Alzheimer's disease as they aged. These findings were published in the *Annals of Neurology* (April, 2006), a journal published by John Wiley & Sons.

Experts theorize that diet may play a role in the development of Alzheimer's disease but epidemiological data on diet and Alzheimer's is conflicting and while individual foods and nutrients have been previously studied, general dietary patterns have not. To address this paucity of data, researchers led by Nikolaos Scarmeas (2006) of Columbia University Medical Center, designed a prospective community-based study of 2,258 non-demented people in New York City. The subjects were part of the Washington Heights-Inwood Columbia Aging project, and for each, the researchers gathered medical and neurological history, did a standardized physical and neurological exam, and conducted an in-person interview to assess health and neuropsychological function. This information was used to diagnose a presence or absence of dementia. Subjects were reassessed approximately every 18 months for an average of 4 years.

The researchers also obtained dietary data from each subject using a semi-quantitative food frequency questionnaire. They determined a Mediterranean Diet score (0-9) based on a previously described method. During the course of the study, 262 members of the study population were diagnosed with Alzheimer's disease. "Higher adherence to the Mediterranean diet was associated with significantly lower risk of developing Alzheimer's disease," the authors report. For each additional point to Mediterranean diet scores (indicating increased adherence to the diet), Alzheimer's risk dropped by 9 to 10 percent. Compared with the subjects in the least adherent group that adhered to a Mediterranean diet the least, subjects in the middle tertile had 15 to 21 percent lower risk of developing Alzheimer's disease, and those in the highest tertile had a 39 to 40 percent lower risk, suggesting a significant dose response effect. The association remained significant even after adjusting for potential confounders such as age, gender, ethnicity, education, caloric intake, BMI, smoking and comorbid conditions.

One possible limitation of this study is the inaccurate measurement of subjects' diets, though the researchers used a previously developed and tested dietary assessment, and suggest that mismeasurements may have actually caused an underestimation of the association. Also, disease misclassification is a possible limitation, though the diagnosis was made by experienced practitioners and was based on comprehensive assessment and standard criteria. Subtle changes in dietary habits as a result of early Alzheimer's symptoms, although another potential limitation, did not seem to be the case since adherence to the Mediterranean diet was found to be quite stable. "We **conclude that higher adherence to the Mediterranean diet is associated with a reduction in risk for Alzheimer's disease,**" they say. In addition, they say that the beneficial effects of the Mediterranean diet for non-neurological conditions have been previously shown to be generalizable to different populations, and that the current study

provided the opportunity to examine the effect of this diet for a neurological disease in a multiethnic community in the U.S.

The Mediterranean diet included high intake of certain foods:

Fruits including apples, oranges, orange or grapefruit juice, peaches, apricots, plums, and bananas Vegetables including tomatoes, broccoli, cabbage, cauliflower, Brussels sprouts, raw or cooked carrots, corn, yams, spinach, collard greens, and yellow squash Legumes including peas, lima beans, lentils, and beans Cereals including cold breakfast cereals, white or dark bread, rice, pasta, and potatoes (baked, broiled, or mashed) Monounsaturated fatty acids, such as those found in olive oil. The Mediterranean diet also includes moderate amounts of fish of all types, low intake of meat and poultry, low to moderate amounts of dairy products, and a moderate amount of alcohol (usually wine served at meals).

Mad Cow Disease and Alzheimer's — Is there a connection?

(<http://www.medicalnewstoday.com/youropinions.php?opinionid=11677>)

Biochemist Colm Kelleher speculates that the infectious "prion" proteins that cause Mad Cow Disease and its brain-wasting human variant, Creutzfeldt-Jakob Disease (CJD), could be a factor in the substantial increase in cases of Alzheimer's disease in recent years. His book Brain Trust (2006) is a medical detective story that traces the origin and spread of the deadly infectious prions that cause Mad Cow disease as they jumped species and ended up in America's food supply. It also shows how human Mad Cow disease is hidden in the current epidemic of Alzheimer's Disease. However according to the " BSE ammonia- magnesium theory " (www.bse-expert.cz) there can be a "no infectious connection" ; see following "BSE and Alzheimer's relationships" about this theory

1.The origin of BSE according to the alternative „ammonia- magnesium theory“

There is the possibility that **hyperammonemia plus hypomagnesaemia „simultaneous“ action** have a strong influence on the CNS, especially in ruminants (Mg absorption in the rumen, especially), so that the BSE has its roots in a more common nutritional problem. This **alternative „ ammonia- magnesium theory“** is based on the chronic Mg-deficiency potentiated by hyperammonemia in ruminants. As a typical example; the ryegrass staggers is showed in ruminants. So, various clinical symptoms can be observed because the nervous system controlling both voluntary and involuntary muscles is affected (Mg and Ca disturbances).

It seems, that **during the chronic hypomagnesemic disease**, the heavy weather changes (cold- rainy, windy...) or nutrition (high intake of crude protein...) stress - these episodes of acute abruptions, may accelerate the nervous, like to „BSE“ disease. If the BSE is involved; a longer- chronic action of corresponding biochemical changes in the blood (CSF) is necessary, to rise **irreversible neurodegenerative changes**. Early of prion diseases, neurons develop intracytoplasmic vacuoles. As the disease progresses, **vacuolization** becomes more pronounced and advanced cases show neuron loss, gliosis (**astrocytosis**), and brain atrophy.

Cellular prion protein (PrPC) is associated with regulation of intracellular free calcium levels through an interaction with voltage-sensitive calcium channels. Toxic effects displayed by **PrPSc (scrapie prion protein) can be blocked by antagonists of N-methyl-D -aspartate (NMDA) receptor channels.**

An important consequence of NMDA receptor activation is the influx of Ca^{2+} into neurons. Overstimulation of the NMDA receptor as well as other excitatory amino acid receptors results in neurotoxicity and neuronal injury. These receptors are considered as the final common pathway for many acute and chronic neurologic conditions.

Studies have demonstrated that **Mg^{2+} can protect against NMDA- induced neurodegeneration**, brain injury, and convulsions. Mg^{2+} competes with calcium at voltage-gated calcium channels both intracellularly and on the cell surface membrane. Mg^{2+} is capable of blocking NMDA receptors both intracellularly and extracellularly.

While non-ruminants absorb Mg primarily from the small intestine, **ruminants are able to absorb much of their Mg requirement from the rumen.** As the dietary protein is readily fermentable, it leads to increased intraruminal ammonia and is normally detoxified in the liver to urea. However, a high rate and extent of degradation of crude protein causing high concentrations of ammonia – N in rumen results in hyperammonemia, (because of diminished capacity of liver to synthesize urea in ornithine cycle), and ruminal ammonia contribute to decreased Mg absorption. It seems to me that there is **the beginning about the „BSE vicious circle“** (see Fig.1).

a/ Action of the hyperammonemia

Ammonia is a main factor in the pathogenesis of hepatic encephalopathy (HE), the CNS is most sensitive to the toxic effects of ammonia. Acute ammonia toxicity is mediated by activation of NMDA receptors. In this process of neuronal death is known that the rise of intracellular Ca^{2+} is an essential step. A rapid increase in ammonia- acute exposure to ammonia; results in an increase in pH_i (intracellular alkalinization) in all cell types, including astrocytes. This results in cytosolic alkalinization (pH action) and leads to calcium-dependent glutamate release from astrocytes. Intracellular alkalinization is accompanied with an increase in $(\text{Ca}^{2+})_i$ in neurons.

During ammonia intoxication, NMDA receptors are excessively stimulated, resulting in a larger influx of Ca^{2+} than usual into neurons. This would elicit a cascade of reactions and eventually lead to neuronal cell death. It has been shown that NH_4^+ induced depolarization in cultured rat cortical astrocytes. This **ammonia-induced depolarization** could also take place in neuronal membranes and result in removal of Mg^{2+} that normally blocks the NMDA receptor channel, leading to excessive activation of the NMDA receptor.

So, the effects of ammonia may be responsible for the reduced astrocytic uptake of neuronally-released glutamate and high extracellular glutamate levels consistently seen in experimental models of the hepatic encephalopathy (HE).

b/ Action of the Mg- deficit

Under normal conditions of synaptic transmission, the NMDA receptor channel is blocked by Mg^{2+} sitting in the channel and only activated for brief periods of time. **Under pathological conditions**, however, overactivation of the NMDA receptor causes an excessive amount of Ca^{2+} influx into the nerve cell, which then triggers a variety of processes that can

lead to necrosis or apoptosis.. For example **energetically compromised neurons become depolarized** because in the absence of energy they cannot maintain ionic homeostasis; this depolarization **relieves the normal Mg²⁺ block** of NMDA receptor-coupled channels because the relatively positive charge in the cell repels positively-charged Mg²⁺ from the channel pore. Hence, during periods of ischemia and in many neurodegenerative diseases, excessive stimulation of glutamate receptors is thought to occur.

Elevations in extracellular glutamate are not necessary to invoke an **excitotoxic mechanism**. Excitotoxicity can come into play even with normal levels of glutamate if NMDA receptor activity is increased, e.g., when neurons are injured and thus become depolarized (more positively charged); this condition relieves the normal block of the ion channel by Mg²⁺ and thus abnormally increases NMDA receptor activity.

Astrocytes in the brain form an intimately associated network with neurons. They respond to neuronal activity and synaptically released glutamate by raising intracellular calcium concentration Ca²⁺. Ability of most neurotransmitters to increase astrocytic Ca²⁺ levels is firmly established. **Astrocytes regulate neuronal calcium levels through the calcium-dependent release of glutamate**. Astrocytic glutamate release pathway is engaged at physiological levels of internal calcium. Astrocytic glutamate release can be triggered by any ligand that stimulates an increase in Ca²⁺...

2. Overstimulation of the NMDA receptor; the connection between Mad Cow Disease and Alzheimer's?

Glutamate mediates most fast excitatory synaptic transmission in the central nervous system, by activating three subclasses of ionotropic receptors--amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate, and N-methyl-D-aspartate (NMDA). Glutamate receptor activation is necessary for normal sensorimotor control, as well as synaptogenesis and synaptic plasticity , but excessive activity of these receptors can contribute to neuronal death in a variety of neuropathological processes, including ischemia, seizures, and neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson disease and Huntington disease (DiFIGLIA 1990; KOCHLAR et al. 1988; PARK et al. 1988; ROTHSTEIN et al. 1990, 1992; SIMON et al. 1984; SRIVASTAVA et al. 1993; TURSKI et al. 1991). The NMDA-type glutamate receptor is thought to play the critical role in induction of synaptic plasticity as well as cell death because of its voltage-dependent magnesium block, high calcium permeability, and slow deactivation and desensitization (BLISS and COLLINGRIDGE 1993; DINGLELINE et al. 1999).

Overstimulation of the NMDA receptor by glutamate is implicated in neurodegenerative disorders. Accordingly, REISBERG et al. (2003) investigated memantine, an NMDA antagonist, for the treatment of Alzheimer's disease. Antiglutamatergic treatment reduced clinical deterioration in moderate-to-severe Alzheimer's disease, a phase associated with distress for patients and burden on caregivers, for which other treatments are not available.

Persistent activation of NMDA receptor in the central nervous system has been considered to contribute to chronic neurodegeneration in Alzheimer's disease. Memantine is postulated to exert its therapeutic effect through its action as a moderate-affinity, uncompetitive NMDA receptor antagonist (LIPTON, 2006).

a/ Alzheimer's treatment by acetylcholinesterase inhibitors (donepezil, rivastigmine and galantamine)

The Cholinergic system is a system of nerve cells that uses acetylcholine as its neurotransmitter, it is damaged in the brains of people with Alzheimer's. So, people with Alzheimer's have low levels of a neurotransmitter- acetylcholine in their brain. Neurones which contain acetylcholine (cholinergic neurones) are found in an area of the brain called the basal nucleus, as well as in the nearby hippocampus, and the cerebral cortex- all areas involved in memory, learning and thinking. Circuits connecting these regions are powered by acetylcholine, and a lack of the neurotransmitter will impair these mental functions.

Acetylcholinesterase (AChE) is the enzyme that breaks down acetylcholine. Clinical trials show that these drugs can stabilise or improve cognition, global assessment scores, mood and behaviour in people with Alzheimer's disease. Unfortunately, as the disease progresses, there are fewer and fewer cholinergic neurones and so there is less potential for these drugs to work. **Thus the drugs only slow the symptomatic progression of the disease**, and don't alter the underlying disease process (effective for some patients **in the early to middle stages** of AD). Sadly, the drugs do not benefit everyone with Alzheimer's disease and sooner or later everyone will stop responding.

b/ Alzheimer's disease treatment by an NMDA antagonist (memantine)

Alzheimer's disease is the most common form of dementia. Many risk factors have been defined in the literature, and the roles of environmental factors, nutrition, some vitamins and trace elements have been investigated. The role of magnesium (Mg) in dementia and other degenerative disorders has been the focus of increased attention in recent years. **Concentration of Mg affects many biochemical mechanisms, which consist of N-methyl D-aspartate (NMDA) receptor response to excitatory amino acids, stability and viscosity of the cell membrane and toxic effects of calcium.** Mg usage with drugs like memantine, which has an influence via Mg, can be useful in dementia treatment. According to the results of these studies, Mg support can facilitate learning and result in improvement in other symptoms. Memantine use has shown some benefit in moderate-to-severe Alzheimer's disease or for vascular dementia. Mg in the treatment of dementia facilitates learning and contributes to improvement in other symptoms; **used in conjunction with memantine it may serve to increase memantine's symptomatic and neuroprotective effects**, via its influence on NMDARs (OZTURK and CILLIER, 2006).

Glutamate is an 'excitotoxic' neurotransmitter- too much glutamate and the cell excites itself to death. Glutamate is also released by dying nerve cells, possibly setting off a 'chain reaction'. It is thought that overactivity of glutamate almost certainly plays a role in Alzheimer's disease. In a clinical trial, **memantine was found to slow the progress of symptoms in patients with more severe disease- a significant finding, as no other drug has been found to be effective in this patient group.** Memantine is the first drug approved for the **treatment of moderate to severe AD.**

Conclusion

According to the BSE ammonia- magnesium theory , there the origin of BSE is a long-term high protein intake with the coincidence of dietary magnesium -deficiency. It seems that the same can be about the Alzheimer's disease .

Alzheimer's, Parkinson's, Type II Diabetes Are Similar At The Molecular Level

<http://www.sciencedaily.com/releases/2007/04/070430102021.htm>

<http://www.bioinfo-online.net/modules/news/article.php?storyid=297>

Alzheimer's disease, Parkinson's disease, type II diabetes, the human version of mad cow disease and other degenerative diseases are more closely related at the molecular level than many scientists realized, an international team of chemists and molecular biologists reported April 29 2007 in the online version of the journal Nature.

Harmful rope-like structures known as amyloid fibrils, which are linked protein molecules that form in the brains of patients with these diseases, contain a stack of water-tight "molecular zippers," the scientists report.

"We have shown that the **fibrils have a common atomic-level structure**," said David Eisenberg, director of the UCLA–Department of Energy Institute of Genomics and Proteomics, a Howard Hughes Medical Institute investigator and a member of the research team. "All of these diseases **are similar at the molecular level; all of them have a dry steric zipper**. With each disease, a different protein transforms into amyloid fibrils, but the **proteins are very similar at the atomic level**."

The research, while still preliminary, could help scientists develop tools for diagnosing these diseases and, potentially, for treating them through "structure-based drug design," said Eisenberg, a UCLA professor of chemistry and molecular biology.

The researchers, including scientists with the European Synchrotron Radiation Facility in Grenoble, France, report 11 new three-dimensional atomic protein structures, including those for both of the main proteins that form amyloid fibrils in Alzheimer's disease.

"It has been a joy to see so many new structures," said Michael Sawaya, a research scientist with UCLA and the Howard Hughes Medical Institute and a member of the team. "Each one is like a Christmas present. Now that we have so many of these that we can classify, I am thrilled to see each three-dimensional arrangement of atoms, what the structural similarities and differences are, and which of the differences are significant. We see many similarities, but there are details that are different. As we study more structures, we expect to determine the common features among them.

"It is clear from the positions of the atoms where the zipper is," Sawaya added. "Like pieces in a jigsaw puzzle, they have to fit together just right. We are finding out how they fit together. We don't yet know all the ways of forming the zippers; we are working to fill in the missing pieces and are hopeful of doing so. Thanks to our colleagues in Grenoble and Copenhagen, technology is not limiting us."

In an earlier Nature paper (June 9, 2005), Eisenberg and his colleagues reported the three-dimensional structure of an amyloid-like protein from yeast that revealed the surprising molecular zipper.

"In 2005, we were like prospectors who found flakes of gold in a stream," Eisenberg said. "Now we see the real nuggets. In this paper, we present atomic-level structures for crystals related to fibrils from proteins associated with numerous human diseases."

The research shows that **very short segments of proteins are involved in forming amyloid fibrils**; Eisenberg and his colleagues know some of the segments. Knowing the segments makes it easier to design tests to detect whether a new drug is effective, Eisenberg noted. Several proteins contain more than one amyloid fibril-forming segment.

"It's exciting how rapidly this work is progressing," said Rebecca Nelson, a UCLA senior postdoctoral fellow with the UCLA-DOE Institute of Genomics and Proteomics and a member of the team. "Once we formed the collaboration with the scientists in France to use the European Synchrotron Radiation Facility, everything became easier."

Nelson describes the proteins associated with Alzheimer's and other amyloid fibril diseases as "transformer" proteins that instead of doing their normal work start forming pathological fibril structures.

Eisenberg's research team used a sophisticated computer algorithm to analyze proteins known to be associated with human diseases. Magdalena Ivanova, a senior research scientist, found that when the computer algorithm says a protein will form an amyloid fibril, the protein almost always does.

While the molecular zipper is very similar in all cases, there are differences, which are cataloged in this Nature paper. For example, while the amyloid fibrils are all characterized by a "cross-beta X-ray diffraction pattern" in a small section of the protein that the scientists call the spine, and there are always two sheets, the sheets can be face to face, or face to back.

If the molecular zipper is universal in amyloid fibrils, as Eisenberg believes, is it possible to pry open the zipper or prevent its formation?

Melinda Balbirnie, a UCLA postdoctoral scholar and a member of the research team, is able to produce fibrils and has developed a test, using a wide variety of chemical compounds, to determine whether the fibrils break up. She is "hopeful" her strategy will succeed in breaking up the fibrils.

A mystery on which the new Nature paper sheds light is what causes different strains of prions (infectious proteins) in which the protein sequence is identical.

"Our research gives a strong hypothesis that the **origin of prion strains is encoded in the packing of the molecules in the fibrils which we are seeing in the crystals**," Ivanova said.

Parkinson's Disease

What is Parkinson's Disease?

Parkinson disease affects both men and women in almost equal numbers. It shows no social, ethnic, economic or geographic boundaries. In the United States, it is estimated that 60,000 new cases are diagnosed each year, joining the 1.5 million Americans who currently

have Parkinson disease. While the condition usually develops after the age of 65, 15% of those diagnosed are under 50.

Parkinson's disease is a neurological disease resulting from damage to the nerves in the area of the brain that is responsible for controlling muscle tension and movement - the basal ganglia. The damaged cells are the ones needed to produce the neurotransmitter called dopamine. The disease usually begins as a slight tremor of one hand, arm, or leg. In the early stages the tremors are more apparent while the person is at rest, such as while sitting or standing, and are less noticeable when the hand or limb is being used. A typical early symptom of Parkinson's disease is "pill-rolling," in which the person appears to be rolling a pill back and forth between the fingers. As the disease progresses, symptoms often get worse. The tremors and weakness affect the limbs on both sides of the body. The hands and the head may shake continuously. The person may walk with stiff, shuffling steps. In many cases, the disease causes a permanent rigid stooped posture and an unblinking, fixed expression.

The cause of Parkinson's disease is unknown, but it is thought that a neurotoxin causes oxidative damage to the basal ganglia in the brain. The basal ganglia controls muscle tension and movement. In the **oxidative damage model, oxidation reactions lead to the generation of free radicals that are capable of destroying the cell membranes and nerve cells.**

What dietary factors are important in Parkinson's Disease?

At this point in time, Parkinson's disease is best treated with drug therapy along with key dietary, nutritional, and herbal recommendations can be used to enhance the effectiveness of drug therapy. The **key dietary strategy is to follow a low protein diet** to enhance the action of L-dopa therapy. **This simple dietary recommendation has been demonstrated to be extremely helpful in several clinical studies and is now a well-accepted supportive therapy.** The usual recommendation is to eliminate good sources of dietary protein from breakfast and lunch (i.e., keep daytime protein intake below 7 grams). This simple dietary practice can offer an effective method for the reduction of tremors and other symptoms of Parkinson's disease during waking hours.

Serum Cholesterol Levels and the Risk of Parkinson's Disease

Several recent findings suggest a role of lipid and cholesterol metabolism in the pathogenesis of Parkinson's disease. Therefore, the authors examined the association between serum levels of cholesterol and the risk of Parkinson's disease in the prospective, population-based Rotterdam Study among 6,465 subjects aged 55 or more years with repeated in-person examination and on average 9.4 years of follow-up (1990-2004). **Higher serum levels of total cholesterol were associated with a significantly decreased risk of Parkinson's disease** (age- and sex-adjusted hazard ratio per mmol/liter increase in cholesterol = 0.77, 95% confidence interval: 0.64, 0.94), with evidence for a dose-effect relation. The association was restricted to women and remained unchanged after adjustment for multiple potential confounders. These findings may indicate a role of lipids in the pathogenesis of Parkinson's

disease. Alternatively, they could reflect the strong correlation--especially in women--between levels of serum cholesterol and the antioxidant coenzyme Q10. If confirmed, this would provide further support for an important role of oxidative stress in the pathogenesis of Parkinson's disease (deLAU et al., 2006).

NMDA glutamate receptors are a class of excitatory amino acid receptors, which have several important functions in the motor circuits of the basal ganglia, and are viewed as important targets for the development of **new drugs to prevent or treat Parkinson's disease (PD)**. NMDA receptors are ligand-gated ion channels composed of multiple subunits, each of which has distinct cellular and regional patterns of expression. They have complex regulatory properties, with both agonist and co-agonist binding sites and regulation by phosphorylation and protein-protein interactions. They are found in all of the structures of the basal ganglia, although the subunit composition in the various structures is different. NMDA receptors present in the striatum are crucial for dopamine-glutamate interactions. The abundance, structure, and function of striatal receptors are altered by the dopamine depletion and further modified by the pharmacological treatments used in PD. In animal models, **NMDA receptor antagonists are effective antiparkinsonian agents** and can reduce the complications of chronic dopaminergic therapy (wearing off and dyskinesias). Use of these agents in humans has been limited because of the **adverse effects associated** with nonselective blockade of NMDA receptor function, but the development of more potent and selective pharmaceuticals holds the promise of an important new therapeutic approach for PD (HALLETT and STANDAERT, 2004).

Schizophrenia

History about “dopamine and gluconate” hypotheses of schizophrenia

For the last several decades, thinking in this field has been **dominated by the hypothesis that hyperfunction of dopamine pathways played a key role in schizophrenia**. However, the therapeutic agents developed from this hypothesis have a slow onset of action and **tend to improve only the positive symptoms of the disease**. The classical "dopamine hypothesis of schizophrenia" postulates a **hyperactivity of dopaminergic transmission at the dopamine D₂ receptor** in the mesencephalic projections to the limbic striatum (SNYDER et al., 1974; CARLSSON, 1988). This hypothesis remains the preeminent neurochemical theory, despite several limitations (DUNCAN et al., 1999). The notion was initially supported by a tight correlation between the therapeutic doses of conventional antipsychotic drugs and their affinities for the D₂ receptor (SEEMAN, 1987; MIYAMOTO et al., 2001). In addition, indirect dopamine agonists (e.g., L -dopa, cocaine, and amphetamines) can induce psychosis in healthy subjects and, at very low doses, provoke psychotic symptoms in schizophrenics (CARLSSON, 1988).

The dopamine hypothesis has received support from postmortem and positron emission tomography (PET) indications of **increased dopamine D₂ receptor levels in the brains of schizophrenic patients** (WONG et al., 1986). However, it has been suggested that upregulation of D₂ receptor expression may be the result of adaptation to antipsychotic drug treatment rather than a biochemical abnormality intrinsic to schizophrenia. In fact, some PET studies show no significant difference in D₂ receptors densities between neuroleptic-naive

schizophrenics and healthy controls (NORDSTROM et al., 1995). There is emerging evidence for a presynaptic dopaminergic abnormality in schizophrenia, implying dysfunction in presynaptic storage, vesicular transport, release, reuptake, and metabolic mechanisms in mesolimbic dopamine systems (LARUELLE et al., 1999). It has been further hypothesized that dysregulation and hyper-responsiveness of presynaptic dopamine neurons could lead to lasting consequences through the induction of sensitization and/or oxidative stress (LEWIS and LIEBERMAN, 2000; LARUELLE, 2000). On the contrary, the **functional activity of dopamine may be decreased in the neocortex in schizophrenia**, which could be, at least partially, associated with negative symptoms (e.g., emotional or cognitive impairment) (LEWIS and LIEBERMAN, 2000).

However, the existence of **anatomical and functional interrelationships between dopamine and glutamate systems** in the central nervous system suggests that inhibition of the NMDA subtype of glutamate receptor (NMDA-R) would influence dopamine neurotransmission (MIYAMOTO et al., 2001; VASILADIS et al., 1999; ZHENG et al., 1999). For example, **NMDA-R antagonists** decrease corticofugal inhibition of subcortical dopamine neurons (CARLSSON et al., 2000), and **thereby enhance the firing rate of dopamine neurons** (ZHANG et al., 1992; MURASE et al., 1993). In humans, PET studies of dopamine receptor occupancy after acute administration of ketamine suggest that the NMDA-R antagonists increase dopamine release in the striatum (BREIER et al., 1998; SMITH et al., 1998; VOLLENWEIDER et al., 2000). In contrast, chronic administration of NMDA-R antagonists **elicits decreased dopamine release** (BREIER et al., 1998) or hypoactivity of dopamine in the prefrontal cortex (JENTSCH and ROTH, 1999). KAPUR and SEEMAN (2002) have shown that both PCP and ketamine have direct effects on D₂ and 5-HT₂ receptors. It has also been proposed that NMDA-R antagonists can cause an excess compensatory release of glutamate that can overactivate unoccupied non-NMDA glutamate receptors, including α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate receptors (MOGHADDAM et al., 1997). Noncompetitive antagonism of NMDA receptors by the open channel blockers is known to induce changes throughout the brain. **NMDA blockade causes an increase in dopamine release in the midbrain and prefrontal cortex** (BUBSER et al., 1992). NMDA blockade also **causes activation of 5HT systems** specifically targeting the 5HT_{1A} receptor (LOSCHER et al., 1990).

NMDA receptor antagonists are induce a state of called “dissociative anesthesia”, which is marked by catalepsy, amnesia, and analgesia (PENDER, 1971). Ketamine and other NMDA receptor antagonists are most frequently used in conjunction with diazepam as anesthesia in cosmetic or reconstructive plastic surgery (ERSEK, 2004), and in the treatment of burn victims (CEBER and SALIHOGU, 2006). Ketamine is a favored anesthetic for emergency patients with unknown medical history because it depresses breathing less than other anesthetics (HESHMATI et al., 2003). The NMDA receptor antagonist dextromethorphan is one of the most commonly used cough suppressants in the world (EQUINOZZI and ROBUSCHI, 2006). NMDA receptor antagonists sometimes induce “psychomimetic” side effects, symptoms resembling psychosis. Such side effects caused by NMDA receptor inhibitors include hallucinations, paranoid delusions, confusion, difficulty concentrating, agitation, alterations in mood, nightmares (MUIR and LEES, 1995), catatonia (AARTS and TYMIANSKI, 2003), ataxia (KIM et al., 2002), anaesthesia (KRISTENSEN et al., 1992) and learning and memory deficits (ROCKSTROH et al., 1996). Because of these psychotomimetic effects, NMDA receptor antagonists, especially phencyclidine, ketamine, and dextromethorphan, are used as recreational drugs. At subanesthetic doses, these drugs have mild stimulant effects, and at higher doses, begin induce dissociation and hallucinations

(LIM, 2003). Several drugs have been found that lessen the risk of neurotoxicity from NMDA receptor antagonists, such as anticholinergics, diazepam, barbiturates (OLNEY et al., 1991).

The NMDA receptor hypofunction (NRH) hypothesis was initially proposed in 1980, by researchers who had found significantly low levels of the neurotransmitter glutamate (Glu) in cerebrospinal fluid. It wasn't until the later 1980's, however, when the results of several studies showed the ability of psychotomimetic agents phencyclidine (PCP) to block NMDA receptors, that the potential association of NRH to schizophrenia was realized. The idea of a **glutamatergic abnormality in schizophrenia was first proposed by Kim, Kornhauber, and colleagues in 1980 (KIM et al., 1980)** based on their findings of low cerebrospinal fluid (CSF) glutamate levels in patients with schizophrenia.

In conclusion; decreased NMDA-R function may be a predisposing or causative factor in schizophrenia (JAVITT and ZUKIN, 1991; COYLE, 1996; JENTSCH and ROTH, 1999). Depressed NMDA-R function is associated with an array of negative symptoms. For example, NMDA-R hypofunction that occurs as the brain ages may be partially responsible for memory deficits associated with aging (NEWCOMER and KRYSTAL, 2001). Schizophrenia may also have to do with inadequate NMDA receptor function (the "glutamate hypothesis" of schizophrenia) (LIPINA et al., 2005).

1. Treatment with NMDA receptor antagonists produces psychosis and schizophrenia

Antagonists of the NMDA subtype of glutamate receptor are of considerable interest for various neurotherapeutic purposes, including preventing neuronal degeneration in stroke and CNS trauma, suppressing neuropathic pain and preventing the development of tolerance to opiate analgesics. Unfortunately, NMDA antagonists can cause potentially serious side effects, including acute neurodegenerative changes in corticolimbic regions of the adult rat brain and psychotic reactions in adult humans. Recreational use or investigator administration of a single low dose of an **NMDA receptor antagonist such as phencyclidine (PCP) or ketamine and the powerful NMDA antagonist, MK-801 - produces "schizophrenialike" symptoms** in healthy individuals and profoundly exacerbates preexisting symptoms in patients with schizophrenia (see following works);

a/ It was found (JAVITT and ZUKIN, 1991) that PCP-induced psychotomimetic effects are associated with submicromolar serum concentrations of PCP. At these concentrations PCP interacts selectively with a specific binding site (PCP receptor) that is associated with the N-methyl-D-aspartate (NMDA)-type excitatory amino acid receptor. Occupation of its receptor by PCP induces noncompetitive inhibition of NMDA receptor-mediated neurotransmission. Other NMDA antagonists such as the dissociative anesthetic ketamine induce PCP-like neurobehavioral effects in proportion to their potency in binding to the PCP receptor and inducing NMDA receptor inhibition. **CONCLUSIONS:** These findings suggest that endogenous dysfunction of NMDA receptor-mediated neurotransmission might contribute to the pathogenesis of schizophrenia. The relative implications of the PCP and amphetamine models of schizophrenia are discussed in relationship to the diagnosis and etiology of schizophrenia (JAVITT and ZUKIN, 1991).

b/ Ketamine, a phencyclidine hydrochloride derivative, is a dissociative anesthetic and a noncompetitive antagonist of the NMDA subtype of excitatory amino acid receptor. KRYSTAL et al (1994) found that ketamine (1) produced behaviors similar to the positive

and negative symptoms of schizophrenia; (2) elicited alterations in perception; (3) impaired performance on tests of vigilance, verbal fluency, and the Wisconsin Card Sorting Test; (4) evoked symptoms similar to dissociative states; and (5) preferentially disrupted delayed word recall, sparing immediate recall and postdistraction recall. **CONCLUSIONS:** These data indicate that NMDA antagonists produce a broad range of symptoms, behaviors, and cognitive deficits that resemble aspects of endogenous psychoses, particularly schizophrenia and dissociative states (KRYSTAL et al., 1994).

c/ Agents that block the NMDA subtype of glutamate receptor induce a schizophrenialike psychosis in adult humans and injure or kill neurons in several corticolimbic regions of the adult rat brain. Susceptibility to the psychotomimetic effects of the NMDA antagonist, ketamine is minimal or absent in children and becomes maximal in early adulthood. **FARBER et al. (1995) examined the sensitivity of rats at various ages to the neurotoxic effects of the powerful NMDA antagonist, MK-801.** Vulnerability was found to be age dependent, having onset at approximately puberty (45 days of age) and becoming maximal in early adulthood. This age-dependency profile (onset of susceptibility in late adolescence) in the rat is similar to that for ketamine-induced psychosis or schizophrenia in humans. These findings suggest that NMDA receptor hypofunction, the mechanism underlying the neurotoxic and psychotomimetic actions of NMDA antagonists, may also play a role in schizophrenia (FARBER et al., 1995).

d/ **MALHOTRA et al. (1997) administered subanesthetic doses of the NMDA receptor antagonist ketamine** in a double-blind, placebo-controlled design to 13 neuroleptic-free schizophrenic patients to investigate if schizophrenics will experience an exacerbation of psychotic symptoms and cognitive impairments with ketamine. They also examined whether schizophrenics experienced quantitative or qualitative differences in ketamine response in comparison to normal controls. Schizophrenics experienced a brief-ketamine-induced exacerbation of positive and negative symptoms with further decrements in recall and recognition memory. They also displayed greater ketamine-induced impairments in free recall than normals. Qualitative differences included auditory hallucinations and paranoia in patients but not in normals. These data indicate that ketamine is associated with exacerbation of core psychotic and cognitive symptoms in schizophrenia. Moreover, ketamine may differentially affect cognition in schizophrenics in comparison to normal controls (MALHOTRA et al., 1997).

e/ Phencyclidine (PCP) and ketamine, both potent non-competitive **antagonists of the NMDA subtype of glutamate receptor (NMDA-R), induce schizophrenia-like symptoms in healthy individuals and worsen some symptoms in schizophrenia** (HIRAYASU et al., 2001; HAZLETT et al., 1999).

In addition, **ethanol is also an antagonist of the NMDA glutamate receptor** (PETRAKIS et al., 2004); what is about the schizophrenia induction in alcoholic individuals?

2. NMDA receptor hypofunction in schizophrenia and hyperfunction in epilepsy

NMDA receptors might have a lower affinity for glycine, explaining why administration of exogenous glycine-agonists results in a favorable clinical response in schizophrenia . Additionally, one could imagine that these receptors might be **less sensitive to glutamate, and, perhaps, more sensitive to Mg²⁺ block** (an area of the open channel close to where PCP

acts). The end result would be a receptor that would function poorly under normal conditions. This imagined receptor has properties virtually opposite to those of NMDAR. Therefore, an understanding of the molecular basis of NMDAR might have direct relevance to the putative **NMDA hypofunction in schizophrenia**. A speculative hypothesis based on this idea is that an altered balance between kinase (enzymes that phosphorylate proteins) and phosphatase (enzymes that dephosphorylate proteins) activity occurs in the pathological state. For example, **in kindling (epilepsy) the receptor might be hyperphosphorylated** (enhancing receptor activity), and **in schizophrenia the receptor might be hypophosphorylated** (diminishing receptor activity). To further speculate, sensitization involving NMDA receptor plasticity may occur during the development of schizophrenia as it does in the development of kindled seizures using similar (but opposing) signal transduction pathways. Attractive candidate proteins that might be involved in the development of kindling and/or schizophrenia include yotiao (a scaffold protein associated with the NMDA receptor), and type I protein phosphatase (PP1) and protein kinase A (PKA), both of which are associated directly with yotiao (and thus with the NMDA receptor), **regulating receptor function via phosphorylation state of the receptor** (WESTPHAL et al., 1999).

For example, although it is proposed that NMDA **receptor hypofunction in schizophrenia and hyperfunction in kindling** might have a common molecular substrate (phosphorylation state of the NMDA receptor), the chains of events leading to these NMDA receptor alterations might be very different. These differences might include relative weight of initiating neurotransmitter activities (e.g., dopamine vs. glutamate), cell populations affected, pattern of gene expression, specific phosphatases and kinases involved, and overall network strengthening/weakening. Regardless of these potential differences, the knowledge of the molecular processes leading to NMDA receptor hyperfunction in kindling would be of great utility in the generation of testable hypotheses for the NMDA receptor hypofunction model of schizophrenia (as well as other psychiatric diseases conceptualized as sensitization processes).

Furthermore, in vitro studies of tissue slice preparations (examining the effects of NMDA receptor antagonism on seizure-like activity) have demonstrated that NMDA receptor-mediated neurotransmission can contribute to **epileptiform activity** (DINGLELINE et al., 1990). A correlate to the role of NMDA receptors in these in vitro models is the role of this receptor subtype in seizure expression in vivo. Several studies have shown that NMDA receptor antagonists are effective antiseizure drugs in models of acute seizures, including chemical and electroshock-evoked seizures (CROUCHER et al., 1982; CLINESCHMIDT et al., 1982). Additionally, **NMDA receptor antagonists** have been shown to be effective antiseizure drugs in **genetic models of epilepsy** (MITROVIC et al., 1990; PATEL et al., 1990; MITROVIC et al., 1991; CHAPMAN et al., 1991; SARRO and SARRO, 1993).

Kindling is a form of experimental epilepsy in which periodic electrical stimulation of a brain pathway induces a permanently hyperexcitable state. Kindling enhances the sensitivity of hippocampal CA3 pyramidal cells to NMDA, consistent with a greater expression of NMDA receptors. Kindling provokes the expression by CA3 pyramidal cells of NMDA receptors with reduced affinity for competitive antagonists (NADLER et al., 1994). The expression of the NMDA receptor component of the excitatory postsynaptic potential (EPSP) may arise from alterations intrinsic to the NMDA receptor itself. Whole-cell patch clamp and single-channel recordings of acutely dissociated dentate granule cells have disclosed increases in mean channel open time as well as a **reduced sensitivity to Mg²⁺ of NMDA receptors following kindling** (KOHR et al., 1993). They have also demonstrated that kindling results in an enhanced potency of NMDA at NMDA receptors on these dentate granule cells (KOHR

and MODY, 1994). The data indicate that kindling induces a population of NMDA receptors whose properties would be expected to enhance the response of dentate granule cells to synaptically released glutamate (i.e., to produce an increase in excitability). It appears likely that the NMDA receptor alterations identified by electrophysiological analysis of single channels on granule cells contribute to the expression of the NMDA receptor-mediated component of the granule cell EPSP following kindling.

NMDA receptor hypofunction and schizophrenia; recent science findings

Schizophrenia is a serious mental disorder that **affects up to 1% of the population worldwide**. As of yet, neurochemical mechanisms underlying schizophrenia remain unknown. **To date, the most widely considered neurochemical hypothesis of schizophrenia is the dopamine hypothesis**, which postulates that symptoms of schizophrenia may result from excess dopaminergic neurotransmission particularly in striatal brain regions, along with dopaminergic deficits in prefrontal brain regions. **Alternative neurochemical models of schizophrenia, however, have been proposed involving glutamatergic mechanisms in general and N-methyl-d-aspartate (NMDA) receptors in particular**. A potential role for glutamatergic mechanisms in schizophrenia was first proposed approximately 15 years ago based on the observation that **the psychotomimetic agents phencyclidine (PCP) and ketamine induce psychotic symptoms** and neurocognitive disturbances similar to those of schizophrenia by blocking neurotransmission at NMDA-type glutamate receptors. Since that time, significant additional evidence has accumulated supporting a role for NMDA hypofunction in the pathophysiology of schizophrenia. Clinical challenge studies with PCP and ketamine have confirmed the close resemblance between NMDA antagonist-induced symptoms and neurocognitive deficits and those observed in schizophrenia, and suggest that **NMDA dysfunction may lead to secondary dopaminergic dysregulation** in striatal and prefrontal brain regions. As compared to dopaminergic agents, NMDA antagonists induce negative and cognitive symptoms of schizophrenia, as well as positive symptoms. **Treatment studies with NMDA modulators**, such as glycine, d-serine, and glycine transport inhibitors (GTIs), have yielded encouraging findings, **although results remain controversial**. Finally, genetic linkage and in vivo neurochemical studies in schizophrenia highlight potential etiological mechanisms giving rise to glutamatergic/NMDA dysfunction in schizophrenia (JAVITT, 2007).

Recently, evidence is accumulating that the exclusive **dopamine hypothesis of schizophrenia has to be abandoned**. Instead, a more integrative approach combines different neurotransmitter systems, in which glutamatergic, GABAergic and dopaminergic pathways interact. This paradigm shift coincides with the recognition that, while typical and modern atypical antipsychotic drugs have efficiently controlled the dramatic psychotic symptoms of schizophrenia, their impact on negative and cognitive symptoms is negligible. Indeed, cognitive decline is now believed to represent the core of schizophrenic morbidity and in this context, impairment of glutamate and more specifically **NMDA function is of major importance**. **Given that astrocytes are important in controlling glutamate homeostasis**, it is necessary to assign a significant role to glial-neuronal interactions in the pathophysiology of schizophrenia. Indeed, recent data from several animal and human studies corroborate this notion (KONDZIELLA et al., 2007).

The NMDA receptor antagonist **psychotomimetic agents phencyclidine (PCP)** has been shown to induce the positive, negative and cognitive symptoms of schizophrenia in healthy patients and cause a resurgence of symptoms in stable patients. These observations led to the NMDA receptor hypofunction hypothesis as an alternative theory for the underlying cause of schizophrenia. According to this hypothesis, **any agent that can potentiate NMDA receptor currents has the potential to ameliorate the symptoms of schizophrenia.** To date, NMDA receptor currents can be modulated by either direct action on modulatory sites on the NMDA receptor (i.e., the glycine co-agonist binding site) or indirectly by activation of G-protein coupled receptors (GPCRs) known to potentiate NMDA receptor function (i.e., mGluR5). This review will discuss the NMDA receptor hypofunction hypothesis, the NMDA receptor as an emerging target for the development of novel antipsychotic agents and progress towards in vivo target validation with GlyT1 inhibitors and mGluR5 positive allosteric modulators. Other potential targets for modulating NMDA receptor currents (polyamine sites, muscarinic receptors, etc...) will also be addressed briefly (LINDSLEY et al., 2006).

Accumulating evidence from both genetic and clinico-pharmacological studies suggests that **D-serine, an endogenous coagonist to the NMDA subtype glutamate receptor, may be implicated in schizophrenia (SZ).** Although an association of genes for D-serine degradation, such as D-amino acid oxidase and G72, has been reported, a role for D-serine in SZ has been unclear. In the study (FUJI et al. (2006) identify and characterize protein interacting with C-kinase (PICK1) as a protein interactor of the D-serine synthesizing enzyme, serine racemase (SR). The binding of endogenous PICK1 and SR requires the PDZ domain of PICK1. The gene coding for PICK1 is located at chromosome 22q13, a region frequently linked to SZ. In a case-control association study using well-characterized Japanese subjects, FUJI et al. (2006) observe an association of the PICK1 gene with SZ, which is more prominent in disorganized SZ. Their findings implicating PICK1 as a **susceptibility gene for schizophrenia are consistent with a role for D-serine in the disease.**

Schizophrenia is characterized by disturbances in sensorimotor gating and attentional processes, which can be measured by prepulse inhibition (PPI) and latent inhibition (LI), respectively. Research has implicated dysfunction of neurotransmission at the NMDA-type glutamate receptor in this disorder. LIPINA et al (2005) examined whether **compounds that enhance NMDA receptor (NMDAR) activity via glycine B site, D-serine** and ALX 5407 (glycine transporter type 1 inhibitor), alter PPI and LI in the presence or absence of an NMDAR antagonist, MK-801. Authors concluded; D-Serine and ALX 5407 display similar effects to clozapine in PPI and LI mouse models, suggesting potential neuroleptic action. Moreover, the finding that agonists of NMDARs and clozapine can restore disrupted LI and disrupt persistent LI may point to a **unique ability of the NMDA system to regulate negative and positive symptoms of schizophrenia** (LIPINA et al., 2005).

NMDA receptor hypofunction may play an important role in the pathophysiology of schizophrenia (BEGANY, 2006)

In a symposium entitled, "Not Just Dopamine Any More: Emerging Glutamatergic Therapies for Schizophrenia," **Professor Joseph Coyle from Harvard Medical School, Cambridge, Massachusetts, and Editor of the Archives of General Psychiatry,** described molecular mechanisms that had recently been identified as being of interest in schizophrenia .

Emerging schizophrenia treatments aim to enhance NMDA receptor function

What do the areas of the brain affected by schizophrenia have in common? “They are synaptically connected by glutamatergic neurons,” related Joseph T. Coyle, Jr, MD, at the 157th Annual Meeting of the American Psychiatric Association. Therefore, “one would have to **agree that glutamate must be involved in some way in the pathophysiology of schizophrenia,**” asserted **Dr. Coyle**, the Eben S. Draper Professor of Psychiatry at Harvard Medical School in Boston.

Dr. Coyle reviewed evidence of glutamate’s role in schizophrenia, particularly the contribution of dysfunctional glutamatergic neurotransmission. He suggested that it might be **possible to develop schizophrenia treatments that modify the function of the N-methyl-D-aspartic acid (NMDA) receptor, a glutamate receptor subtype that, when hypofunctional, may account for the deficient glutamatergic neurotransmission associated with schizophrenia.** Dr. Coyle noted that at least five major pharmaceutical companies are already developing drugs that improve schizophrenia symptoms by enhancing NMDA receptor function.

The evidence

The hypothesis that **NMDA receptor hypofunction might be important in schizophrenia** was not given much credence until investigators performed experiments in which healthy volunteers received subanesthetic infusions of the dissociative anesthetic ketamine, a known **NMDA receptor antagonist**. The infusions reproduced the positive and negative symptoms of schizophrenia, including paranoia, thought disorder, loose associations, illusions, emotional withdrawal, and psychomotor retardation.

The volunteers displayed other features of schizophrenia, such as selective neurocognitive impairment, abnormal eye tracking, evoked potential abnormalities, and increased dopamine release in the brain. However, Dr. Coyle pointed out, they scored in the normal range on the Mini- Mental State Examination, as patients with schizophrenia typically do.

Postmortem studies have since found a significant reduction in the activity of glutamate carboxypeptidase (GCP) II, the **enzyme that degrades the endogenous NMDA receptor antagonist N-acetyl-alpha L-aspartyl-L-glutamate (NAAG)**, in the hippocampus, prefrontal cortex, and temporal cortex of patients with schizophrenia compared with controls. “If the enzyme that degrades NAAG is reduced, more of this NMDA receptor antagonist would be available in the brain,” Dr. Coyle reasoned.

Magnetic resonance spectroscopy findings support that hypothesis, showing reduced levels of the byproducts of NAAG metabolism in the brain areas where the decreased GCP II activity occurred. The results of these postmortem studies have been replicated, said Dr. Coyle. Further evidence of NMDA receptor hypofunction in schizophrenia was the discovery of a translocation in the human genome at the location of the GCP II gene. The translocation is associated with an increased schizophrenia risk, and so are four polymorphisms in the enzymes that degrade the NMDA receptor agonist D-serine.

Patients with schizophrenia also appear to carry a mutation in the gene encoding for a protein that regulates the degradation of D-serine, another endogenous agonist at the glycine modulatory site. One of the results of the genetic abnormalities seen in these patients is thought to be a large decline in brain levels of D-serine. **Low levels of D-serine are the best**

evidence to date that brain D-serine levels are reduced in schizophrenia, however, noted Dr. Coyle.

Molecular Mechanisms

Professor Joseph Coyle described molecular mechanisms that had recently been identified as being of interest in schizophrenia. These mechanisms are predominantly glutamatergic, and **he described in some detail the 2 classes of ionotropic glutamate receptors, namely the AMPA/kainate receptors (AMPA) and the n-methyl d-aspartate receptors (NMDAR).**

The AMPAR (GluR 1-4) are the primary mediators of excitatory postsynaptic currents (EPSCs). The NMDAR (NR1; NR2A-D) contribute to the EPSC and play a more fundamental role in coincidence detection. EPSCs and coincidence detection are believed to be important mediators of neuroplasticity in mechanisms such as learning and memory, and these may be disrupted in schizophrenia. At the resting membrane potential, **the NMDAR channel is blocked by Mg^{2+} , which is removed upon depolarization. The NMDAR channels are sufficiently large to readily transduce Ca^{2+} , which activates** the intracellular kinases that ultimately regulate gene expression. The recruitment of NMDAR during high presynaptic glutamatergic activity results in the permanent increase in synaptic efficacy known as long-term potentiation (LTP). **Influx of Ca^{2+} through the NMDAR during LTP causes the recruitment of AMPAR from intracellular stores to the synapse.** Persistent hyperactivity through a glutamatergic pathway can cause sprouting of postsynaptic spines via NMDAR activation, further strengthening synaptic connections. NMDAR activation has trophic effects, especially during development, with inactivity of NMDAR resulting in neuronal apoptosis.

Another unique characteristic of the NMDAR is that, in addition to the binding site for the agonist, glutamate, **there is a glycine modulatory site (GMS) to which glycine and/or d-serine bind.** The GMS needs to be occupied for glutamate to open the channel. The **availability of d-serine depends upon the activities of serine racemase (SR) and the degrading enzyme d-amino acid oxidase (DAAO), whereas the availability of glycine is determined by the activity of the glycine transporter, GlyT-1. Notably, both SR and GlyT1, as well as the glutamate transporters that protect against excitotoxicity (EAAT 1 and 2), are expressed exclusively in astrocytes, indicating a vital role of astroglia in modulating glutamatergic neurotransmission.** Dr. Coyle concluded that **regulation of the availability of glycine/d-serine at the GMS plays a critical role in optimal NMDAR function.**

Indirect stimulation

Assuming that NMDA receptor hypofunction contributes to schizophrenia, **enhancing the NMDA receptor function may decrease symptoms.** The goal, emphasized Dr. Coyle, should be to stimulate the receptor indirectly at the glycine modulatory site. “You would not want to directly activate it because if you overactivate it you will kill neurons,” he warned.

In animal studies, indirect stimulation of the NMDA receptor reversed the effects of NMDA receptor antagonists and enhanced cognition. This strategy has shown promise in double-blind, placebo-controlled clinical trials, as well. These trials focused primarily on **schizophrenia treatment with the full NMDA receptor agonists glycine and D-serine, the partial agonist D-cycloserine, and the glycine transporter-1 agonist N-methylglycine**

Reduced negative symptoms and improved cognition were seen in patients with schizophrenia treated with glycine, Dr. Coyle reported. Three trials of D-cycloserine for schizophrenia found improvement in negative symptoms; one of these trials also showed cognitive improvement. **Decreased negative and positive symptoms and improved cognition were observed in trials of D-serine and N-methylglycine for schizophrenia.**

The negative symptoms of schizophrenia worsened in a dose-finding study in which D-cycloserine, a partial agonist, was added to clozapine. In other trials, adding the full agonists glycine or D-serine to clozapine had no effect on the negative symptoms of schizophrenia. These clinical findings along with the results from animal studies **suggest that clozapine's unique effects on negative symptoms might be due to its effect on the glycine modulatory site on the NMDA receptor.**

Addressing criticism

Critics of schizophrenia treatments that target hypofunctional NMDA receptors have argued that such treatment is unlikely to work, **because NMDA receptors are present throughout the brain, not just in the areas affected by schizophrenia.** However, the positive effect of agonist therapies in some clinical trials **may be attributed to enhanced function of a "discrete subpopulation of NMDA receptors,"** suggested Dr. Coyle.

The NMDA receptors on GABAergic interneurons in the frontal and temporal cortexes and in the hippocampus may be that discrete subpopulation, he speculated, because studies have shown a heightened sensitivity of those receptors to endogenous and exogenous NMDA receptor antagonists. Furthermore, postmortem analysis of the brains of patients with schizophrenia **showed markedly decreased NMDA receptor co-expression on GABAergic interneurons.**

Also, functional studies of the hippocampus in patients with schizophrenia have detected abnormally high levels of neuronal activity. "This level of disinhibition is not sufficient for seizures but certainly is sufficient to impair cognition," remarked Dr. Coyle, "[and it] supports the notion that the excitatory NMDA receptors on these GABAergic interneurons are impaired."

Receptor hypofunction

Dr. Coyle and colleagues proposed that the **NMDA receptors on the GABAergic interneurons of brain areas affected by schizophrenia are hypofunctional.** The results are disinhibition that impairs cortical-hippocampal processing and disruption of the excitatory output sufficient to trigger subcortical dopamine release that leads to psychosis. **"Enhancing glycine modulatory site occupancy by agonists is a plausible treatment for schizophrenia, especially the negative and cognitive symptoms,"** Dr. Coyle maintained. "This strategy ... may be more powerful than just the **symptomatic treatment** that we have been using. By enhancing NMDA receptor function in conjunction with rehabilitation, we may be **able not only to deal with symptoms but perhaps to reverse the cognitive and social deficits** that are the most disabling aspects of this disorder" (BEGANY, 2006).

Cannabinoids are known to inhibit calcium channels- glutamate release in schizophrenia

and to inhibit progression of certain neurodegenerative diseases (by reducing intracellular calcium release)

Substantial increases in both prevalence (the percentage of the population affected by the disease) and incidence (the frequency of new occurrences) of the disease are forecast by the end of the decade, with **increases in schizophrenia starting earlier among young men in particular**. The research study matches historic trends in cannabis use and exposure from a national population survey against estimates of new occurrences of schizophrenia in three English cities (Nottingham, Bristol and the London Borough of Southwark). The researchers assess what might happen to **schizophrenia cases if we assume a causal link between cannabis use and onset of psychotic symptoms**, an association widely recognised by some psychiatrists and researchers and considered recently by the Advisory Council on the Misuse of Drugs. Exposure to cannabis grew fourfold over the thirty years to 2002, and its use among under-18s by 18-fold, say the researchers. If cannabis use causes schizophrenia, these increases in its use would lead to increases in overall schizophrenia incidence and prevalence of 29 per cent and 12 per cent respectively, between 1990 and 2010. If cannabis causes schizophrenia - and that remains in question - then by 2010 up to 25 per cent of new cases of schizophrenia in the UK may be due to cannabis, according to a new study by Dr Matthew Hickman of the University of Bristol and colleagues, published in *Addiction* journal.

Cannabinoids are naturally occurring compounds in vertebrates, and are known to play an important role in intercellular signaling. The chemical **tetrahydrocannabinol (THC) found in marijuana is a cannabinoid**, though different from the ones produced by the body. Trends of cannabis use among adolescents in the USA indicate that cannabis use under the age of 16 years is a fairly new phenomenon that has appeared only since the early 1990s (JOHNSTON et al., 2002). One would therefore predict an increase in rates of schizophrenia in the general population over the next 10 years. Indeed, there is already some evidence that the incidence of schizophrenia is currently increasing in some areas of London, especially among young people. BOYDELL et al. (2003) investigated changes in incidence of schizophrenia in Camberwell, south-east London, between 1965 and 1997. There was a **continuous and statistically significant increase in the incidence of schizophrenia, which was greatest in people under 35 years of age** and was not gender-specific. The incidence of schizophrenia has doubled in south-east London over the past three decades (BOYDELL et al., 2003).

So, there is evidence that cannabis use can contribute to schizophrenia. Some studies suggest that cannabis is neither a sufficient nor necessary factor in developing schizophrenia, but that cannabis may significantly increase the risk of developing schizophrenia and may be, among other things, a significant causal factor. Nevertheless, some previous research in this area has been criticised as it has often not been clear whether cannabis use is a cause or effect of schizophrenia. To address this issue, a recent review of studies from which a causal contribution to schizophrenia can be assessed has suggested that cannabis statistically doubles the risk of developing schizophrenia on the individual level, and may, assuming a causal relationship, be responsible for up to 8% of cases in the population (ARSENEAULT et al., 2004). An older longitudinal study (ANDREASSON et al., 1987), suggested six-fold increase of schizophrenia risks for high consumers of cannabis (use on more than fifty occasions) in Sweden.

It is generally accepted that the influx of Ca^{2+} as a result of excessive activation of the NMDA receptor underlies the toxic actions of glutamate in many systems. During periods of ischemia and in many neurodegenerative diseases, excessive stimulation of glutamate receptors is thought to occur. These neurodegenerative diseases, including Alzheimer's

disease, Parkinson's disease, Huntington's disease..., are caused by different mechanisms but may share a final common pathway to neuronal injury due to the overstimulation of glutamate receptors, especially of the NMDA subtype. On the other hand, recently, a hypofunction of glutamatergic neurons has been hypothesized to cause schizophrenia. Functional diversity of NMDA receptors may be expected from the assembly of different subunit combinations, and there is a very important „Ca²⁺-dependent manner” which permits activation of NMDA receptors. So, also dietary calcium deficiency can be important about „NMDA hypofunction“ in schizophrenia...

However, there can be **another example about hypoglutamatergic condition; cannabinoids** are known to inhibit Ca²⁺ channels- glutamate release in schizophrenia. Cannabinoids are a group of terpenophenolic compounds present in Cannabis sativa L. **Natural cannabinoids are only known to occur naturally in the cannabis plant.** The chemical tetrahydrocannabinol (THC) **found in marijuana is a cannabinoid**, though different from the **endogenous cannabinoids** naturally produced in the bodies of animals. The broader definition of cannabinoids refer to a group of substances that are structurally related to THC or that bind to cannabinoid receptors. THC is the primary psychoactive component of the plant, medically, it appears to ease moderate pain and to be neuroprotective. Endocannabinoids serve as intercellular 'lipid messengers', signaling molecules that are released from one cell and activate the cannabinoid receptors present on other nearby cells. So, cannabinoids are naturally occurring compounds in vertebrates, and are known to play an important role in intercellular signaling. In 1992, the first such compound was identified as arachidonoyl ethanolamide and named **anandamide. It has a pharmacology similar to THC**, although its chemical structure is different.

THC mediates the majority of its activities through stimulation of cannabinoid receptors (CB). Two cannabinoid receptors, CB1 and CB2, were discovered only the last ten years. CB1 exists primarily in the central nervous system, while CB2 is found primarily in the peripheral nervous system. Also, endogenous anandamide binds to both the central (CB1) and peripheral (CB2) cannabinoid receptors, and is found in nearly all tissues in a wide range of animals, it is about as potent as THC. Activation of CB1 receptor inhibits neurotransmitter release in many brain regions. CB1 receptors are essentially absent in the medulla oblongata, the part of the brain stem that is responsible for respiratory and cardiovascular functions. Thus, there is not a risk of respiratory or cardiovascular failure as there is with many other drugs. CB1 receptors appear to be responsible for the euphoric and anticonvulsive effects of cannabis.

Activation of CB1 receptor (CB1R) inhibits neurotransmitter release in many brain regions but the mechanism of action is debated (ALGER, 2002). In expression systems and cell bodies, CB1R couples to **activation of K⁺ channels or inhibition of neuronal Ca²⁺ channels, or both** (HOWLETT et al., 2002). Either of these mechanisms can reduce Ca²⁺ influx at nerve terminals and thereby inhibit transmitter release. Activation of K⁺ channels may change the presynaptic action potential (AP) and thus indirectly modulate Ca²⁺ channel activity (DIANA and MARTY, 2003). Alternatively, **CB1R activation may directly inhibit presynaptic Ca²⁺ channels** coupled to exocytosis. Investigations of CB1R action in nerve terminals have relied on measurements of cytosolic Ca²⁺ concentration with indicator dyes (KREITZER and REGEHR, 2001) and occlusion experiments using specific Ca²⁺ channel blockers (SULLIVAN, 1999; ROBBE et al., 2001; WILSON and NICOLL, 2001); however, direct recordings of CB1R-dependent modulation of presynaptic Ca²⁺ currents or APs are still missing. **There is evidence that cannabinoids can regulate glutamate release, oxidant free radicals and calcium influxes** (TWITCHELL et al., 1997; HAMPSON et al., 1998;

KREITZER and REGEHR, 2001; HOWLETT et al., 2002), which, in excess, can cause neuronal death. Cannabinoids can tonically regulate NMDA glutamate receptor activity in vitro and support the in vivo observation that CB₁ regulates NMDA-induced and ischaemic excitotoxicity (NAGAYAMA et al., 1999; PARMENTIER-BATTEUR et al., 2002).

So, communication between the cells requires the release of a glutamate neurotransmitter, triggered by calcium currents passing through a specific calcium channel. **Cannabinoids are known to inhibit calcium channels.** If we shut down the channel, we shut down the release of glutamate, and profoundly alter the cell's ability to signal.

The mechanism of endocannabinoid synaptic transmission is understood by the following events: an excitatory transmission of the neurotransmitter glutamate causes an influx of calcium ions into the post-synaptic neuron. Through a mechanism not yet fully understood, the presence of calcium post-synaptically induces the production of endocannabinoids in the post synaptic neuron. In standard neurotransmission, the pre-synaptic neuron releases neurotransmitter into the synaptic cleft which binds to cognate receptors expressed on the post-synaptic neuron. Upon binding, the neuron depolarizes. This depolarization facilitates in the influx of calcium into the neuron; this increase in calcium activates an enzyme called [[transacylase] which catalyzes the first step of endocannabinoid biosynthesis. **Release of endocannabinoids may occur in response to elevations in intracellular Ca²⁺,** for example during prolonged depolarizations (ALGER, 2002) or during flash photolysis of caged Ca²⁺ (BRENOWITZ and REGEHR, 2003). Endocannabinoid release during mGluR1 activation by mGluRs selective agonist (S)-3,5-dihydroxyphenylglycine (DHPG)), however, may also be Ca²⁺ independent in the cerebellum (MAEJIMA et al., 2001) and hippocampus (CHEVALEYERE and CASTILLO, 2003).

KUSHMERICK et al. (2004) investigated the mechanisms by which activation of group I metabotropic glutamate receptors (mGluRs) and CB₁ cannabinoid receptors (CB₁Rs) leads to inhibition of synaptic currents at the calyx of Held synapse in the medial nucleus of the trapezoid body (MNTB) of the rat auditory brainstem. Their data suggest that activation of postsynaptic mGluRs triggers the **Ca²⁺-dependent release of endocannabinoids** that activate CB₁ receptors on the calyx terminal, which **leads to a reduction of presynaptic Ca²⁺ current and glutamate release.** This observation may suggest a very close coupling between the site of internal Ca²⁺ release (or Ca²⁺ influx) triggered by group I mGluR activation and a Ca²⁺-dependent step in endocannabinoid synthesis and release (KUSHMERICK et al., 2004). So, endocannabinoid release is Ca²⁺ dependent **in the MNTB.**

Exogenously administered cannabinoids are neuroprotective in several different cellular and animal models. **Cannabinoids produce neuroprotection by reducing intracellular calcium release** from ryanodine-sensitive stores (ZHUANG et al., 2005). Their results suggest that cannabinoids prevent cell death by initiating a time and dose dependent inhibition of adenylyl cyclase, that outlasts direct action at the CB₁ receptor and is capable of reducing Ca²⁺_i via cAMP/protein kinase-dependent process during the neurotoxic event. Emerging evidence also indicates that **cannabinoids may play a role in slowing the progression of certain neurodegenerative diseases, such as Multiple Sclerosis, Parkinson's disease, Alzheimer's, and Amyotrophic Lateral Sclerosis (ALS).** There is accumulating evidence in vitro and in vivo to support the hypothesis that the cannabinoid system can limit the neurodegenerative processes that drive progressive disease, and may provide a new avenue for disease control (JACKSON et al., 2005).

Calcium deficiency may be a predisposing or causative factor in NMDAR hypofunction and in schizophrenia

Most of the **better known neurotransmitter systems** - dopamine, noradrenaline, serotonin (5HT), and acetylcholine in particular - **have modulatory roles**. They are produced by a few neurons located in specific clusters, and **drugs affecting them often have specific effects** (recreational or medical, or both). Receptors for these neurotransmitters tend to **operate fairly slowly, taking milliseconds** or longer to communicate. Rather than directly changing the potential of the neuron, **they often trigger second-messenger responses**.

On the other hand, most of the brain's **regular function operates quickly, and involves the excitatory and inhibitory amino acids** (EAAs and IAAs, respectively). The receptors for amino acids are generally ion channels; when the receptor is activated, **ions enter or exit the cell which change its potential**. Most of the **excitatory neurotransmission** in the central nervous system (CNS) is mediated by the **endogenous excitatory amino acids (EAAs)** glutamate, aspartate and homocysteine.

Most of the **endogenous inhibitory neurotransmission is mediated by gamma-aminobutyric acid (GABA)**, about 25% of cortical neurons utilize this **inhibitory neurotransmitter**. Subclasses of GABA neurons differ in their morphological, biochemical, and functional characteristics, indicating that they likely play distinct roles in regulating cortical circuitry. Three subclasses of GABA neurons **express different calcium-binding proteins**. The (a) type of GABA neurons **express parvalbumin (PV)**, and provide inhibitory synapses to the cell bodies of nearby excitatory pyramidal neurons (Py) and to their initial axon segments; these appear to be positioned to regulate the firing of pyramidal neurons.

Excitatory amino acids (EAAs) modulate the firing of almost all neurons in the CNS, as excitatory neurotransmission can result in both neuronal inhibition and excitation. The glutamate system is the best characterised of the EAA systems. Glutamate system play a vital role in the mediation of excitatory **synaptic transmission**. This process is the means by which cells in the brain (**neurons**) communicate with each other. An electrical impulse in one cell causes an influx of calcium ions and the subsequent release of a chemical neurotransmitter (e.g. glutamate). The transmitter diffuses across a small gap between two cells (the **synaptic cleft**) and stimulates (or inhibits) the next cell in the chain by interacting with **receptor** proteins. The specialised structure that performs this vital function is the **synapse** and it is in the synapse that the ionotropic glutamate receptors are generally found. The ionotropic receptors themselves are **ligand gated ion channels**, ie on binding glutamate that has been released from a companion cell, charged ions such as Na^+ and Ca^{2+} pass through a channel in the centre of the receptor complex. This flow of ions results in a **depolarisation** of the plasma membrane and the generation of an electrical current that is propagated down the processes (dendrites and axons) of the neuron to the next in line.

The post-synaptic effects of glutamate are mediated via several receptor subtypes. Different combinations of these subtypes determine the specific functional capability of individual synapses and neurons. Since many combinations of subunits are possible for each type of **glutamatergic receptor the system is extremely complex**. There are **four types of glutamatergic receptor**, each with unique regional and synaptic distributions. They include both the ionotropic and metabotropic receptor families. **Ionotropic receptors** allow the passage of charged ions into the neuron, for example chloride (Cl^-) and calcium (Ca^{2+}).

Glutamatergic receptors are grouped according to similarities in amino acid sequence and pharmacodynamic properties such as affinity for glutamate and threshold for channel opening.

Ionotropic glutamatergic receptors open cation-permeable channels to mediate sodium (Na^+), potassium (K^+) or calcium (Ca^{2+}) ion flow. There are **three families of ionotropic receptors**: the N-methyl-D-aspartate (**NMDA**), the amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (**AMPA**) and the **kainate receptors**.

Metabotropic glutamatergic receptors are activated via G-proteins rather than via cation channels. They have seven transmembrane domains and, being G-protein coupled, they are similar to most dopamine, serotonin and noradrenaline receptors, activating phospholipase C or inhibiting adenylate cyclase. **They are divided into three groups: type I (mGluR1 and 5), type II (mGluR2 and 3) and type III (mGluR4, 6, 7 and 8)**. Each group appears to have specific functions. Type I are post-synaptic, occurring in hippocampal, amygdala and thalamic neurons and, to a lesser extent, in the cortex and ventral striatum. Types II and III are presynaptic modulators of glutamate release. Within type II, mGluR2 is restricted to the cortex and dentate gyrus, whereas mGluR3 appears to be the only metabotropic subtype expressed on glia. **Metabotropic glutamate receptor**, is a newer discovery, and seems to involve **second messenger systems** and produce metabolic effects within the neuron.

1. Activation of AMPA receptors (under Ca^{2+} -dependent manner) permits activation of NMDA receptors

AMPA receptors are predominantly post-synaptic receptors, widely distributed in the cortex and ventral striatum and in temporal lobe structures such as the hippocampus and amygdala, with lower levels in the thalamus. Of the three ionotropic receptors in the CNS, AMPA receptors occur at the greatest density. They include GluR1, GluR2, GluR3 and GluR4. The agonists acting at AMPA receptors are AMPA and amino-3-hydroxy-5-tert-butyl-4-isoxazole propionic acid (ATPA). They mediate most fast excitatory transmissions in the brain. Sustained activation of AMPA receptors **by, for instance, a train of impulses arriving at a pre-synaptic terminal**, depolarises the post-synaptic cell, **releasing the channel inhibition and thus** allowing NMDA receptor activation. **Presence of the glutamate receptor 2 (GluR2) subunit prevents calcium influx through AMPA-receptor complexes. So, unlike GluR2-containing AMPARs, NMDA receptors are permeable to calcium ions as well as being permeable to other ions.** Thus NMDA receptor activation leads to a calcium influx into the post-synaptic cells, **a signal thought to be crucial for the induction of NMDA-receptor dependent LTP (long-term potentiation) and long-term depression (LTD).**

AMPA receptors (AMPA) are both glutamate receptors and cation channels that are integral to plasticity and synaptic transmission at many postsynaptic membranes. One of the most widely and thoroughly investigated forms of plasticity in the nervous system is known as **long-term potentiation (LTP)**. There are two necessary components of LTP: **presynaptic glutamate release, and postsynaptic depolarization**. LTP is an increase in the strength of a chemical synapse that lasts from minutes to several days. It is widely considered one of the **major mechanisms by which memories are formed and stored in the brain**. The mechanisms of early LTP (E-LTP) and late LTP (L-LTP) can be classified into one of three categories: induction, maintenance, and expression.

Induction is the process by which a short-lived signal triggers the phase of LTP to begin. **Induction of E-LTP occurs when the intracellular calcium concentration exceeds a critical threshold in the postsynaptic cell (LYNCH, 2004)**. In many types of LTP, the influx of calcium that drives E-LTP induction **requires the NMDA receptor**, which is why these types of LTP are **considered to be NMDA receptor-dependent (LYNCH, 2004)**. This critical

threshold is defined by **the concentration of calcium required** to transiently activate several protein kinases, including calcium/calmodulin- dependent protein kinase II (CaMKII) and protein kinase C (PKC) (SWEATT, 1999). To a lesser extent, protein kinase A (PKA) and mitogen- activated protein kinase(MAPK) activation also contribute to E-LTP induction (SWEATT, 1999).

While induction entails the transient activation of CaMKII and PKC, maintenance of E-LTP is characterized by their persistent activation. During this stage, CaMKII and PKC lose **their dependence on calcium** and become autonomously active. Consequently they are able to carry out the phosphorylation events that underlie LTP expression (SWEATT, 1999). The **primary event underlying the expression of E-LTP is the phosphorylation of AMPA receptors** to increase the efficiency of synaptic transmission (MALENKA and BEAR, 2004).

The natural progression of E-LTP, L-LTP is induced by changes in gene expression and protein synthesis brought about by the persistent activation of protein kinases such as MAPK and PKA (SWEATT, 1999). These kinases are thought to alter gene expression through the phosphorylation of transcription factors such as **cAMP response element-binding protein (CREB)**. Ambient glutamate in the nerve synaptic cleft activates Ca^{2+} -permeable AMPA receptors on the postsynaptic neurons. This allows an **influx of Ca^{2+} that causes a gradual increase in $[\text{Ca}^{2+}]_i$** ; because of the absence of mGluR-mediated mechanisms for clearing Ca^{2+} . This **increased $[\text{Ca}^{2+}]_i$ activates Ca^{2+} -sensitive adenylate cyclases (AC)**. The cAMP generated by AC activates protein kinase A (PKA). **PKA then directly phosphorylates** (ZIRPEL et al., 2000)

Activation of the transcription factor **calcium/cAMP response element-binding protein (CREB)** is dependent on phosphorylation by protein kinase A (PKA), Ca^{2+} /calmodulin-dependent kinases (CaMKs), or ribosomal S6 kinases (RSKs) (WALTON and DRAGUNOW, 2000). One of the most well characterized signals for CREB phosphorylation and **activation is an increase in $[\text{Ca}^{2+}]_i$** ; (SHENG et al., 1990; WALTON and DRAGUNOW, 2000). Using dynamic calcium imaging, ZIRPEL et al. (2000) showed that treatments that block AMPA receptor activation, or **prevent increases in $[\text{Ca}^{2+}]_i$, also block the phosphorylation of CREB.**

It had been reported that activation of AMPA presynaptic receptors at physiological pH (pH 7.4) **elicits the release of the $[\text{H}^3]$ transmitters under study in an external Ca^{2+} -dependent manner** (PITTALUGA et al., 1987). Hippocampal noradrenergic and serotonergic, striatal dopaminergic and cortical cholinergic nerve endings are endowed with presynaptic receptors of the **AMPA type, whose activation induces a Ca^{2+} -dependent, exocytotic-like release** of noradrenaline, dopamine, 5-HT and acetylcholine (DESCE et al., 1991; FINK et al., 1995; PITTALUGA et al., 1997; GHERSI et al., 2003).

Glutamate receptors of the NMDA type mediating transmitter release exist on rat hippocampal (PITTALUGA and RAITERI, 1990) and cortical (FINK et al., 1990) noradrenergic axon terminals, on striatal dopaminergic nerve endings (ROBERTS and ANDERSON, 1979; KREBS et al., 1991) and on cortical serotonergic nerve endings (FINK et al., 1995). **Release-enhancing AMPA and NMDA receptors were shown to coexist** on the same noradrenergic (PITTALUGA and RAITERI, 1992) or dopaminergic (KREBS et al., 1991) nerve terminal, where **activation of AMPA receptors permits activation of NMDA receptors** in the presence of physiological concentrations of Mg^{2+} .

2. NMDA receptor function and activation

The most structurally complex **glutamatergic receptor is the NMDA receptor (NMDAR)**. This is the glutamate receptor most often implicated in neuropsychiatric disorders. It is an ion channel made up of different and variably assembled protein isoforms. It is the only ionotropic receptor to **control Ca^{2+} conductance** in addition to the conductance of Na^+ and K^+ . When the channel is activated there is an **influx of Na^+ and Ca^{2+} ions and an efflux of K^+ ions. Binding of magnesium (Mg^{2+}) ions** to sites within the channel prevents Ca^{2+} influx. Activation of the channel can occur only if there is **simultaneous glutamate and glycine binding and partial depolarisation (channel is more positively charged)** of the membrane potential. Glycine is an 'obligate co-agonist' for glutamate, i.e. glutamate cannot act on the NMDAR in the absence of glycine. The simultaneous binding of the two transmitters and partial depolarisation permits Mg^{2+} displacement and channel opening. **So, activation of NMDA receptors** requires binding of both glutamate and the co-agonist glycine **for the efficient opening of the ion channel which is a part of this receptor. In addition, a third requirement is** membrane depolarization. **A positive change in transmembrane potential will make it more likely that the ion channel in the NMDA receptor will open** by expelling the Mg^{2+} ion that blocks the channel **from the outside**.

The NMDA receptor is found **predominantly post-synaptically** and seems to be concentrated primarily in the limbic system, **co-localised with AMPA receptors**. The **voltage dependence of NMDA receptors** has the effect of enhancing the depolarisation initiated by non-NMDA receptor channels. **Ca^{2+} can subsequently act as a second messenger and initiate a wide range of intracellular responses** that underlie a number of complex neurophysiological phenomena. NMDA receptor activity is under the influence of several factors, and the receptors have several different regulatory sites of interest. NMDA receptors include

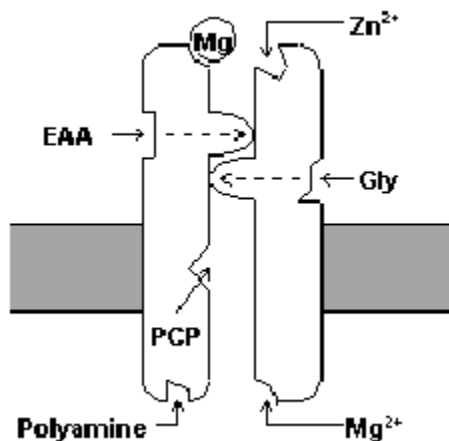
NMDA receptors are composed of assemblies of **NR1 subunits (isoforms A–G) and NR2 subunits (isoforms A–D), which can be one of four separate gene products (NR2A–D)**. Expression of both subunits are required to form functional channels- so, the glutamate binding domain is formed at the junction of NR1 and NR2 subunits (hence the need for both subunits to be expressed). The ion channel regulated by these receptors is **blocked by phencyclidine (PCP), ketamine and the NMDA analogue MK–801**. Potent direct-acting agonists at the NMDA receptors are NMDA and glutamate. In addition to NMDA receptor; the glycine binding site is found on the NR1 subunit .

The **NR2B subunit also possesses a binding site for polyamines**, regulatory molecules that modulate the functioning of the NMDA receptor. At resting membrane potentials, NMDA receptors are inactive. This is due to a **voltage-dependent block of the channel pore by magnesium ions**, preventing ion flows through it . NMDA-R hypofunction in the cortical association pathways could be responsible for a variety of cognitive and other negative symptoms (CARLSSON et al., 2000) and, in mice, **partial deletion of the NMDA-R1 (NR1) subunit causes the same behavioral abnormalities as PCP** (MOHN et al., 1999). In addition, the NR1 hypomorphic animals manifest reduced [^{14}C]-2-deoxyglucose uptake in the medial prefrontal and anterior cingulate cortices, as is observed in chronic schizophrenic patients (DUNCAN et al., 2002).

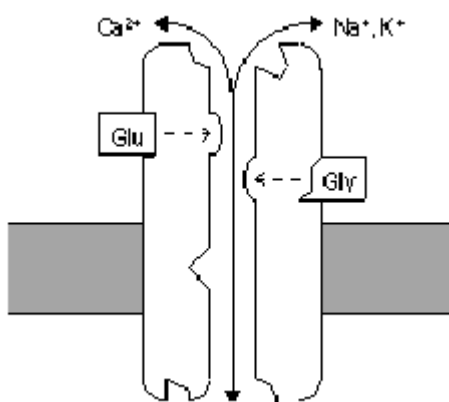
The NMDA receptor has seven distinct binding sites. Three of these are located on the **exterior surface of the cell**, two are located **on the cell interior**, one on the **inside of the**

channel, and one (the **magnesium ion site**) is **present both on the inside and outside surfaces**. There are two agonist sites on the exterior are the cell, denoted EAA and glycine (Gly); they correspond to the excitatory amino acids (glutamate and aspartate) and glycine. Both sites must be occupied before the channel can open enough for any ions to pass through. A third site is the target of zinc ions (Zn^{2+}), which block the channel when present.

The exterior of the channel contains a magnesium ion site. This site is also present on the inside of the cell (alternatively, it may be located within the channel itself). A magnesium ion normally occupies the exterior site; the interior site is probably empty under biological conditions. The interior of the cell contains two binding sites. One binds to polyamines (spermine and spermidine). Occupancy of one of the polyamine sites relieves tonic proton block and, thus, **potentiates NMDA receptor activation in a pH-dependent manner**. At higher concentrations, however, polyamines act on an extracellular site to produce a voltage-dependent block of the ion channel and, thus, inhibit receptor activation (see figure below; "NMDA channel");



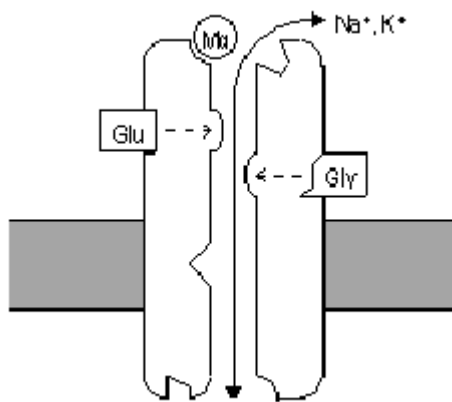
The other, is a **phosphorylation site**. Enzymes can bind to this site and enhance or reduce the activity of the receptor. Finally, inside the channel itself is the PCP₁ site, where PCP, ketamine, MK-801 (dizocilpine), DXM, and dextrorphan all bind. **The channel must be fully open for these drugs to enter**; once in place they "clog up" the channel (see; "Fully open NMDA channel");



NMDA receptors are unique for several reasons. Unlike most receptors, they require two agonists (glutamate or aspartate, plus glycine) before the channel opens. These two agonists (glutamate and glycine) bind to two different locations on the NMDA receptor. After both

agonists have bound to the channel, it opens enough for potassium to enter, and the receptor operates much like AMPA and kainate receptors. Normally, **a magnesium ion** is bound to a specific location at the opening of the channel; **this ion allows potassium to pass through but prevents calcium**, possibly due to its size. This binding is due to electrostatic forces.

Once the cell becomes activated enough, however, the cell potential rises enough that the **magnesium ion is no longer stuck** to the cell. **Calcium can enter** (and exit, although this doesn't happen) **the cell through the fully open NMDA channel**. Once inside, calcium sets into motion a series of responses which enhance the strength of the synapse. **Calcium only enters the cell when the membrane potential is low enough and when the synapse is activated**. If the neuron is only slightly active, the NMDA channel may open partially, but the magnesium ion won't get a chance to leave its binding site (see figure below; "Partially open NMDA channel");



However, if the neuron should be rapidly or substantially activated, **the magnesium ion will be released, and calcium can enter the cell, enhancing synaptic strength**. This process of enhancing synaptic strength, called "Long-Term Potentiation" (LTP), is one of the mechanisms by which neurons can change their functioning and "learn".

As mentioned above, the NMDA receptor is permeable to calcium as well as to sodium and potassium (MacDARMOT et al., 1986; MAYER and WESTBROOK, 1987; ASCHER and NOWAK, 1988). In addition to gating by glutamate and glycine, **NMDA receptor activation is voltage-dependent secondary to a block of the channel pore by Mg^{2+} at more negative membrane potentials** (NOWAK et al., 1984; JAHR and STEVENS, 1990). Because of this voltage-dependent block, **NMDA receptor activation occurs only when the neuron is partially depolarized (more positively charged)**, perhaps following activation of AMPA receptors (FAGG and MASSIEU, 1991). The influx of calcium through this receptor subtype and the fact that its activation is coupled to depolarization (i.e., activity-dependent) have implicated the NMDA receptor in neuronal plasticity, both in normal development and in neuropathology.

When glutamate and glycine bind and the cell is depolarized to remove Mg^{2+} block, the NMDA receptor channel opens with **consequent influx of Ca^{2+} and Na^+ into the cell**, the amount of which can be altered by higher levels of agonists and by substances binding to one of the modulatory sites on the receptor. The two modulatory sites are the magnesium (Mg^{2+}) site within the ion channel and an S-nitrosylation site located toward the N terminus (and hence extracellular region) of the receptor (LIPTON, 2004). The findings, derived from recombinant cotransfection/coexpression experiments, suggest that **native NMDA receptors**

may differ in their sensitivity to voltage-dependent Mg²⁺ block, agonists, and antagonists as a function of their subunit composition. Thus, functional diversity of native NMDA receptors may be expected from the assembly of different subunit combinations (KRAUS et al., 1994).

The permeation pathway of NMDA receptors has a property that sets them apart from other conventional ligand-gated receptors. At hyperpolarized membrane potentials more negative than about -70 mV, the **concentration of Mg²⁺ in the extracellular fluid of the brain is sufficient** to virtually abolish ion flux through NMDA receptor channels even in the presence of the coagonists glutamate and glycine (NOWAK et al., 1984).

NMDA receptor channel complex contributes to excitatory synaptic transmission at sites throughout the brain and the spinal cord, and is modulated by a number of endogenous and exogenous compounds. NMDA receptors play a key role in a wide range of physiologic and pathologic processes. Glutamate is in the glutamate binding site and glycine is in the glycine binding site. Allosteric sites that would cause inhibition of the receptor are not occupied. NMDARs require the binding of two molecules of glutamate or aspartate and two of glycine (LAUBE et al., 1997).

Activation of NMDA receptors requires binding of glutamate or aspartate (aspartate does not stimulate the receptors as strongly (PHILIP et al., 2005). In addition, NMDARs also require the binding of the co-agonist glycine for the efficient opening of the ion channel which is a part of this receptor. **D-serine has also been found to co-agonize the NMDA receptor with even greater potency than glycine.** D-serine is produced by serine racemase in astrocyte cells and is enriched in the same areas as NMDA receptors. Removal of D-serine can block NMDA mediated excitatory neurotransmission in many areas. Recently, it has been shown that D-serine is also synthesized in neurons, indicating a role for neuron-derived D-serine in NMDA receptor regulation.

NMDA receptor function is also strongly regulated by chemical reduction and oxidation, via the so-called "redox modulatory site." Through this site, reductants dramatically enhance NMDA channel activity **while oxidants either reverse the effects of reductants or depress native responses.** It is generally believed that NMDA receptors are modulated by endogenous redox agents such as glutathione, lipoic acid and the essential nutrient pyrroloquinoline quinone (AIZENMAN et al., 1989).

The ion channels coupled to classical NMDA receptors are generally the most permeable to Ca²⁺. Excessive activation of the NMDA receptor in particular leads to production of damaging free radicals and other enzymatic processes contributing to cell death (LIPTON and ROSENBERG, 1994; LIPTON and NICOTERA, 1998).

A positive change in transmembrane potential will make it more likely that the ion channel in the NMDA receptor will open by expelling the Mg²⁺ ion that blocks the channel from the outside. This property is fundamental to the role of the NMDA receptor in memory and learning, and it has been suggested that this channel is a biochemical substrate of Hebbian learning, where it can act as a coincidence detector for membrane depolarization and synaptic transmission. The NMDA receptor is modulated by a number of **endogenous and exogenous compounds. Mg²⁺ not only blocks the NMDA channel** in a voltage-dependent manner but also potentiates NMDA-induced responses at positive membrane. **Magnesium treatment has been used to produce rapid recovery from depression** (EBY and EBY, 2006). Na⁺, K⁺, and Ca²⁺ not only pass through the NMDA receptor channel but also modulate the activity of NMDA receptors. Zn²⁺ blocks the NMDA current in a noncompetitive and a voltage-independent manner. It has been demonstrated that polyamines do not directly activate NMDA receptors, but instead act to potentiate or inhibit glutamate-mediated responses.

3. Astrocytes can release glutamate in a calcium-dependent manner

Astrocytes, a subtype of glial cells, have numerous characteristics that were previously considered exclusive for neurons. One of these characteristics is a **cytosolic Ca²⁺ oscillation that controls the release of the chemical transmitter glutamate** and atrial natriuretic peptide. These chemical messengers appear to be released from astrocytes **via Ca²⁺-dependent exocytosis**. Glutamate can be released from astrocytes, and several mechanisms have been proposed. Glutamate has been demonstrated to be an important signaling molecule for neuron-glia communication. **Astrocytes express receptors and transporters for glutamate** and recently have also been demonstrated to contain the protein machinery necessary to release glutamate by exocytosis through vesicles (BEZZI et al., 2004) and a fusion-related mechanism (ZHANG et al., 2004; KREFT et al., 2004). Overall, astrocytes have many characteristics that were previously considered exclusive for neurons and are therefore **actively involved in cell signaling by releasing glutamate**. Astrocytic glutamate release is calcium-dependent and can be triggered by any ligand that stimulates an increase in Ca²⁺, such as bradykinins (PARPURA et al., 1994), prostaglandins (BEZZI et al., 1998). **Even a spontaneous Ca²⁺_i increase leads to glutamate release from astrocytes** (PASTI et al., 2001).

The evidence obtained during the last few years has established a **new concept of the synaptic physiology**, the tripartite synapse, in which **astrocytes play an active role** by exchanging information with the synaptic elements (ARAQUE et al., 1999; CARMIGNOTO, 2000; AULD and ROBITABILE, 2003; NEWMAN, 2003). This concept is based on the demonstration that astrocytes display a form of **excitability based on intracellular Ca²⁺ variations** (PASTI et al., 1997; VERKHRATSKY et al., 1998; HAYDON, 2001; NEDERGAARD et al., 2003), **respond to synaptically released neurotransmitters** (PORTER and McCARTHY, 1996; PASTI et al., 1997; GROSCHE et al., 1999; LATOUR et al., 2001; ARAQUE et al., 2002), and modulate neuronal excitability and synaptic transmission by releasing neuroactive substances through, at least some of them, **Ca²⁺-dependent mechanisms** (ARAQUE et al., 1998a, 1998b; KANG et al., 1998; NEWMAN and ZAHS, 1998; ROBITAILLE, 1998; PARRI et al., 2001; BEATTIE et al., 2002; BROCKHAUS and DEITMER, 2002; NEWMAN, 2003; ZHANG et al., 2003; FIACCO and McCARTHY, 2004; LIU et al., 2004).

The ability of astrocytes to release glutamate through a **Ca²⁺-dependent mechanism** is well established (BEZZI et al., 1998, 2004; ARAQUE et al., 2000; PARPURA and HAYDON, 2000; PASTI et al., 2001; ZHANG et al., 2004). On the other hand the **ability of most neurotransmitters to increase astrocytic Ca²⁺ levels is firmly established** (PORTER and McCARTHY, 1997; VERKHRATSKY et al., 1998). Recent reports have shown that astrocytic receptor activation by exogenously applied transmitters **may have synergistic effects that increase the Ca²⁺ signal** (FATATIS et al., 1994; CORMIER et al., 2001; SUL et al., 2004). Ca²⁺ elevations in astrocytes stimulate the release of glutamate, which acting on presynaptic or postsynaptic receptors **modulates synaptic transmission and neuronal excitability** (ARAQUE et al., 1998a, 1998b; KANG et al., 1998; PARRI et al., 2001; PASTI et al., 2001; BROCKHAUS and DEITMER, 2002; FIACCO and McCARTHY, 2004; LIU et al., 2004).

4. The availability of D-serine and glycine transporter GlyT-1; depends on Ca²⁺ concentration

D-Serine, recently appreciated as the endogenous ligand for the glycine site of the glutamate NMDA receptor, overturns fundamental axioms of biology as well as those of neuroscience. It is a D-amino acid, and it is synthesized and stored in glia rather than neurons.

MOTHET et al. (2000) showed that selective degradation of D-serine with D-amino acid oxidase greatly attenuates NMDA receptor-mediated neurotransmission as assessed by using whole-cell patch-clamp recordings or indirectly by using biochemical assays of the sequelae of NMDA receptor-mediated calcium flux. The inhibitory effects of the enzyme are fully reversed by exogenously applied D-serine, which by itself did not potentiate NMDA receptor-mediated synaptic responses. Thus, **D-serine is an endogenous modulator of the glycine site of NMDA receptors** and fully occupies this site at some functional synapses (MOTHET et al., 2000).

Released glutamate acts on receptors on the protoplasmic astrocytes closely apposed to the synapse to release D-serine, which **coactivates postsynaptic NMDA receptors together with glutamate**. D-Serine is formed by **serine racemase (enzyme)**, which directly converts L-serine to D-serine. **Inhibitors of this enzyme should reduce NMDA neurotransmission** and might be therapeutic in stroke and other conditions associated with glutamate excitotoxicity (SNYDER and FERRIS, 2000).

These researchers also found **D-serine and serine racemase concentrated in astrocytes adjacent to NMDA receptors**, but less common or nonexistent in other neural tissues. For years, neuroscientists assumed that NMDA receptors could only be stimulated by a single neurotransmitter, an amino acid called glutamate. They **now know that two neurotransmitters are needed to stimulate the NMDA receptors**. D-serine was recently proposed by Hopkins scientists (Baltimore University) as the second, largely because microscope images of tagged D-serine show it's **physically near NMDA receptors in the synapse**. Also, knocking D-serine out with enzymes quickly stops NMDA receptors from being active.

COOK et al. (2002) found that divalent cations such as **calcium or manganese were necessary for complete serine racemase (SR) enzyme activity**, whereas the presence of chelators such as EDTA completely inhibited the enzyme. Moreover, direct binding of calcium to SR was evidenced using $^{45}\text{Ca}^{2+}$. Treatment of astrocytes with the calcium ionophore as well as with compounds that augment the intracellular calcium levels such as glutamate or kainate led to an increase in the amount of D-serine present in the extracellular medium. These results suggest that there might be a **glutamatergic-mediated regulation of SR activity by intracellular Ca^{2+} concentration** (COOK et al. 2002).

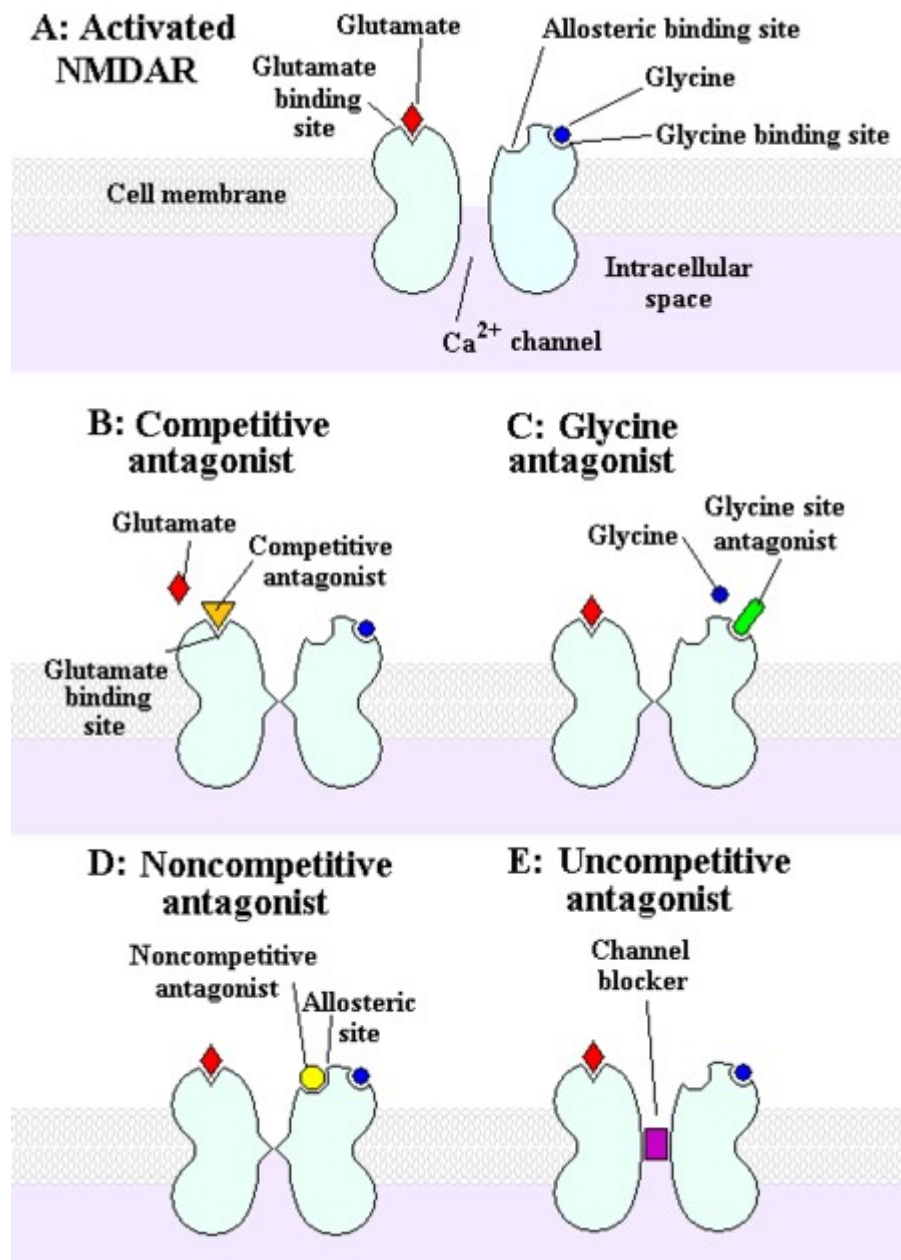
In addition, when the availability of D-serine depends upon the activities of serine racemase (SR), the availability of glycine is determined by the activity of the glycine transporter, GlyT-1. In the vertebrate CNS, glycine acts as an obligatory coagonist of glutamate at NMDA receptors. This role depends on extracellular glycine levels, regulated by Na^+/Cl^- -dependent transporters GLYT-1, present mainly in glial cells, and GLYT-2, predominantly neuronal. LOPEZ et al. (2005) show that high affinity transport by GLYT-1 is regulated by calcium from intracellular stores. Notably, both SR and GlyT1, as well as the glutamate transporters that protect against excitotoxicity, are expressed exclusively in astrocytes, indicating a vital role of astroglia in modulating glutamatergic neurotransmission.

5. „Calcium deficiency“ can intensify NMDA receptor blockade

Why? Increasing intracellular Ca^{2+} attenuates the NMDA -receptor antagonist-mediated loss of GAD67 and parvalbumin PV immunoreactivity in cortical PV interneurons...

It has long been known that treatment with **NMDA receptor antagonists** produces psychosis and cognitive deficits that are reminiscent of the clinical picture of schizophrenia and these data led to the **NMDA receptor hypofunction model of schizophrenia**. There simplified model of NMDAR activation and **various types of NMDAR blockers**;

A: To open, an NMDAR must bind glutamate and glycine, and must not be bound by inhibitors that can cause the NMDAR to close by **binding to allosteric sites**. NMDAR antagonists fall into four categories: **competitive antagonists** (B), which bind to and block the glutamate binding site; **glycine antagonists** (C), which bind to and block the glycine site; **noncompetitive antagonists** (D), which inhibit NMDARs by binding to allosteric sites; and **uncompetitive antagonists** (E), which block the ion channel by binding to a site within it (KIM et al., 2002).



Different drugs inhibit NMDA receptors in different ways. **Competitive antagonists** block sites to which the neurotransmitter glutamate binds and activates receptors. Similarly, glycine antagonists block the site to which glycine binds to activate NMDA receptors. **Noncompetitive antagonists** (memantine...) prevent the NMDA receptor from activating by binding to **allosteric sites**, whereas **uncompetitive antagonists** (dizocilpine ; MK- 801, ketamine, nitrous oxide, phencyclidine...) physically block the channel in the NMDA receptor through which ions flow by occupying it. **Allosteric regulation** is the regulation of an enzyme or protein by binding an effector molecule at the protein's allosteric site (that is, a site other than the protein's active site). Effectors that enhance the protein's activity are referred to as allosteric activators. Noncompetitive antagonism of NMDA receptors by the open channel blockers is known to induce changes throughout the brain. **NMDA blockade causes an increase in dopamine release in the midbrain and prefrontal cortex** (BUBSER et al., 1992). NMDA blockade also causes activation of 5HT systems specifically targeting the 5HT1A receptor (LOSCHER and HONACK, 1993).

6. NMDA receptor blockade reduces the number of calcium-binding protein PV-immunoreactive neurons

Increasing intracellular Ca^{2+} attenuates the NMDA -receptor antagonist-mediated loss of parvalbumin (PV) immunoreactivity in cortical PV interneurons.

In animals, NMDA receptor blockade has been found to **reduce the number of PV-immunoreactive neurons in the entorhinal cortex** (CUNNINGHAM et al., 2006); this reduction is accompanied by robust disruption in γ rhythms. Interestingly, these effects may be mediated primarily by the NR2A subunit. Hypofunction of NMDA receptors on GABA neurons, perhaps especially those that contain PV, directly disrupts the synchronization of neural circuits in the γ frequency range by altering inhibitory control of pyramidal cell networks. **GABAergic (GABA = γ -aminobutyric acid) neurons** from different brain regions contain **high levels of parvalbumin (PV)**, both in their soma and in their neurites. **Parvalbumin is a slow Ca^{2+} buffer** that may affect the amplitude and time course of intracellular Ca^{2+} transients in terminals after an action potential, and hence may regulate short-term synaptic plasticity.

Recently there has been a considerable amount of interest in the role that **gamma (γ)-band EEG oscillations** might play in the **cognitive abnormalities that characterize schizophrenia**. WOO et al (2006) suggest, that reduced glutamatergic inputs onto the fast-spiking GABA cells that contain the **calcium-binding protein parvalbumin (PV)** via NMDA receptors, perhaps **especially those that contain the NR2A subunit**, may mediate, at least in part, the well-reported **downregulation of the 67 kD isoform of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD)67** and PV and the **disruption of γ band synchrony in schizophrenia**.

Several lines of evidence suggest that a **hypoglutamatergic condition** may induce a phenotypic **loss of cortical parvalbumin (PV)-positive GABAergic interneurons**, such as that observed in brain tissue of schizophrenic subjects. However, **it is not known whether the loss of PV interneurons is a consequence of the hypoglutamatergic condition or a secondary aspect of the disease** (KINNEY et al., 2006).. **Their results suggest that the activity of NR2A-containing NMDA receptors play a pivotal role in the maintenance of the GABAergic function of PV interneurons.**

A large contribution of NMDA receptors to subthreshold calcium signals and synaptic excitation was demonstrated in parvalbumin (PV) interneurons (GOLDBERG et al., 2003). **Increasing intracellular Ca^{2+} attenuates the NMDA-receptor antagonist-mediated loss of GAD67 and PV immunoreactivity** in cortical PV interneurons

When present in cortical interneurons, **calcium-binding proteins are assumed to play a role in maintaining calcium homeostasis** and to modulate neuronal excitability and resistance to biochemical damage. Parvalbumin, which displays a slow binding and dissociation rate for Ca^{2+} , has been proposed to act as a slow calcium buffer **regulating intracellular calcium homeostasis** (LEE et al., 2000) and to be involved in the regulation of presynaptic calcium signaling and neurotransmitter release (COLLIN et al., 2005).

Expression of the 67 kDa form of glutamic acid decarboxylase 67 (**GAD67**), **the enzyme responsible for most of GABA synthesis in the brain**, and expression of the **calcium-binding protein parvalbumin (PV)** were shown to be consistently decreased in the prefrontal cortex of **schizophrenic subjects** (ASADA et al., 1996; MIRNICS et al., 2000; REYNOLDS et al., 2001; HASHIMOTO et al., 2003; TORREY et al., 2005). This reduction occurred in the PV-positive subset of interneurons, a key GABAergic system responsible for the control of cortical output (LEWIS et al., 2005).

Calcium homeostasis may be particularly important in PV interneurons, and **calcium entering through NMDA and calcium-permeable AMPA receptors may play a fundamental role** in the synaptic activation of these interneurons (GOLDBERG et al., 2003).

7. Increase in pH_i (intracellular alkalization) results in NMDA receptor overactivation (excitotoxicity)

The activity of NMDA receptors is strikingly sensitive to the changes in H^+ concentration, and partially inhibited by the ambient concentration of H^+ under physiological conditions. The frequency of NMDA receptor channel openings is **reduced by protons over the physiological pH range**, with a midpoint at pH 7.4, such that at pH 6.0 receptor activation is suppressed nearly completely (NOWAK et al., 1984).

A rapid increase in ammonia (high protein intake...) results in an increase in pH_i (intracellular alkalization) in all cell types, including astrocytes, and **NMDA receptors are excessively stimulated**, resulting in a larger influx of Ca^{2+} than usual into neurons. It is commonly known that ammonia (NH_4^+/NH_3) application induces an increase in pH_i in many different cell systems. How does ammonia cause excessive activation of NMDA receptors? It has been shown that NH_4^+ induced depolarization in cultured rat cortical **astrocytes** (ALLERT et al., 1998). **This ammonia-induced depolarization** could also take place in neuronal membranes and result in removal of Mg^{2+} that normally blocks the NMDA receptor channel, leading to excessive activation of the NMDA receptor (FELIPO and BUTTERWORTH, 2002).

So, **under these pathological conditions**; overactivation of the receptor causes an excessive amount of Ca^{2+} influx into the nerve cell, which then triggers a variety of processes that can lead to necrosis or apoptosis. Apoptotic-like excitotoxicity is caused in part by excessive stimulation of the NMDA subtype of glutamate receptor. When activated, the NMDA receptor opens a channel that allows Ca^{2+} (and other cations) to move into the cell. Elevations in extracellular glutamate are not necessary to invoke an excitotoxic mechanism.

Excitotoxicity can come into play even with normal levels of glutamate if NMDA receptor activity is increased, e.g., **when neurons are injured and thus become depolarized** (more positively charged); this condition relieves the normal block of the ion channel by Mg^{2+} and thus abnormally increases NMDA receptor activity (ZEEVALK and NICKLAS, 1992).

Since NMDA receptors (NMDAR) are one of the most harmful factors in excitotoxicity, antagonists of the receptors have held much promise for the treatment of conditions that involve excitotoxicity, including traumatic brain injury, stroke, and neurodegenerative diseases. Energetically compromised neurons become depolarized (more positively charged) because in the absence of energy they cannot maintain ionic homeostasis; this depolarization relieves the normal Mg^{2+} block of NMDA receptor-coupled channels because the relatively positive charge in the cell repels positively-charged Mg^{2+} from the channel pore. Hence, during periods of ischemia and in many neurodegenerative diseases, excessive stimulation of glutamate receptors is thought to occur. These **neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease...**, are caused by different mechanisms but may share a final common pathway to neuronal injury due to the **overstimulation of glutamate receptors**, especially of the NMDA subtype (LIPTON and ROSENBERG, 1994).

8. CONCLUSIONS

Recently, evidence is accumulating that the exclusive **dopamine hypothesis of schizophrenia has to be abandoned** because significant additional evidence has accumulated supporting a **role for NMDA hypofunction in the pathophysiology of schizophrenia**. Clinical challenge studies with NMDA antagonist have confirmed the close resemblance between NMDA antagonist-induced symptoms and neurocognitive deficits and those observed in schizophrenia, and suggest that **NMDA dysfunction may lead to secondary dopaminergic dysregulation** in striatal and prefrontal brain regions. NMDA receptors on the GABAergic interneurons of brain areas affected by schizophrenia are „NMDA hypofunctional“. **Activation of the NMDA receptor channel can occur only** if there is simultaneous glutamate and glycine binding and partial depolarisation of the membrane potential. However, treatment studies with NMDA modulators, such as glycine, D-serine, and glycine transport inhibitors (GTIs), have yielded encouraging findings, although results remain controversial. Why? Because- perhaps, NMDA receptors might have a lower affinity for glycine, explaining why administration of exogenous glycine-agonists results in a **favorable clinical response in schizophrenia**. Additionally, one could imagine that these **receptors might be less sensitive to glutamate**, and, perhaps, more sensitive to Mg^{2+} block. So, **NMDA receptors may differ in their sensitivity to voltage-dependent Mg^{2+} block**, agonists, and antagonists as a function of their subunit composition.

Thus, functional diversity of NMDA receptors may be expected from the assembly of different subunit combinations, and there is very **important „Ca²⁺-dependent manner” which permits activation of NMDA receptors** ... So, **dietary calcium deficiency** can be important about „NMDA hypofunction“ in schizophrenia... Calcium in cells is tightly regulated and mostly unrelated to necessary dietary calcium. **However, a low content of calcium in the ration decreases the magnesium requirements of the animal**. The lower the calcium level in the animal- human ration (and in the tissue cells); the more marked is **„NMDA receptors blocking effect of Mg^{2+} “**; both intracellularly and extracellularly.

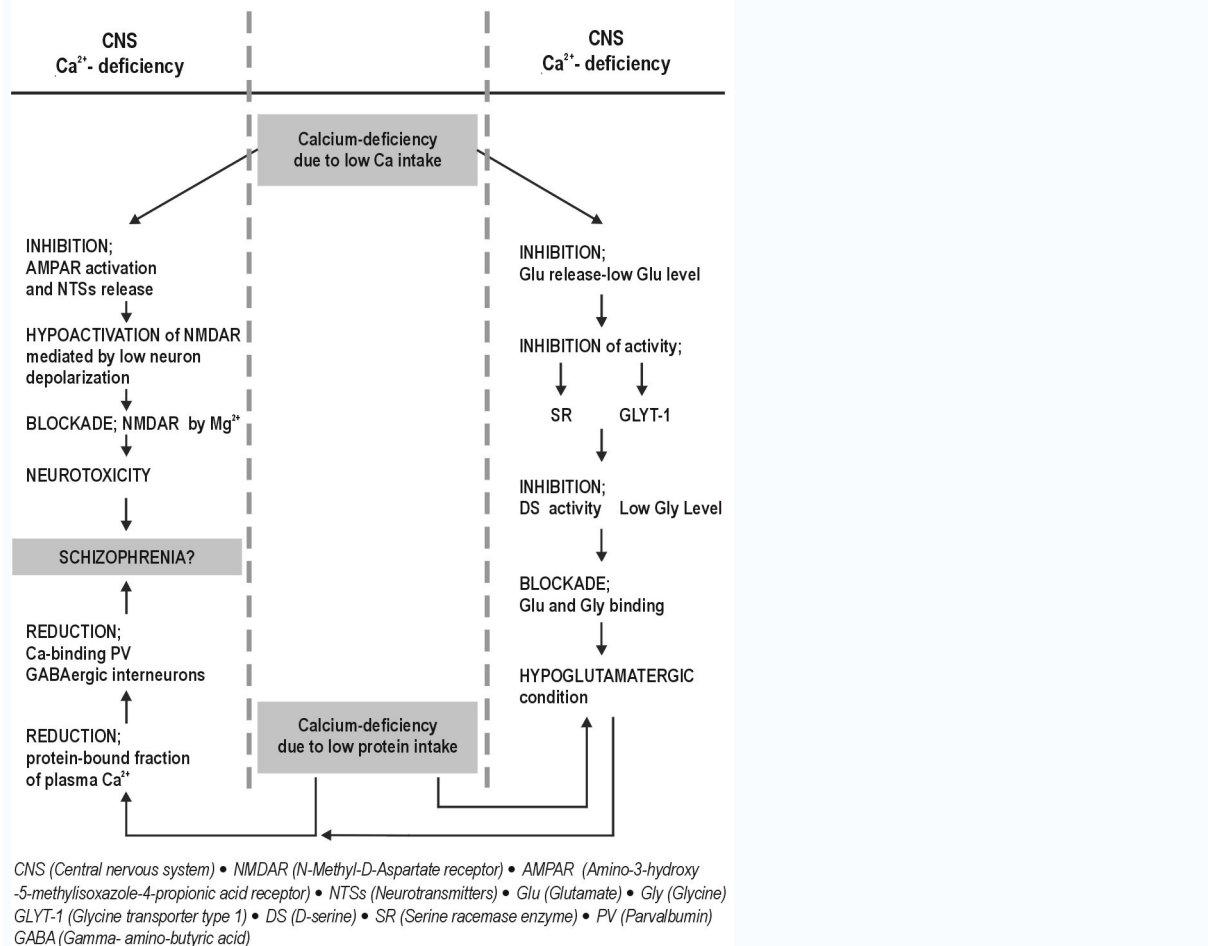
Maintenance of the body calcium stores depends mainly on dietary Ca intake, and on absorption of Ca from the gastrointestinal (GI) tract. For example, the majority of Americans do not get enough calcium in their diet- nearly 75 percent of women and 50 percent of men according to the United States Department of Agriculture (USDA). And only 14 percent of teen girls and 35 percent of teen boys are meeting the recommended dietary allowance. This deficit is a crippling statistic considering how critical calcium is to the body's infrastructure. But now with the innovation of a double-calcium fluid milk, every cookie dunk, spoonful of cereal and breakfast smoothie can provide twice the nutrient that can help reduce the risk of osteoporosis, keep teeth strong, battle high blood pressure and may even aid weight loss as part of a reduced calorie diet (Medical News Today, 2004). In addition, diets moderate in protein (in the approximate range of 1.0 to 1.5 g protein/kg) are associated with normal calcium metabolism. At low protein intakes, intestinal calcium absorption is reduced, resulting in increases in serum PTH; as induced secondary hyperparathyroidism (KERSTETTER et al., 1998; 2003). Hypoproteinemia is associated with a decrease in total calcium, hypoproteinemia can reduce the protein-bound fraction of plasma calcium. Calcium is an important component of a healthy diet. Calcium supplements are used to prevent and to treat calcium deficiencies. There are conflicting recommendations about when to take calcium supplements. However, most experts agree that no more than 500 mg should be taken at a time because the percent of calcium absorbed decreases as the amount of calcium in the supplement increases. It is recommended to spread doses throughout the day, with the last dose near bedtime. Recommended daily calcium intake varies from 1000 to 1500 mg, depending upon the stage of life. Calcium plays a vital role in the physiology and biochemistry of organisms and of the cell, particularly in signal transduction pathways. The amount of total calcium varies with the level of serum albumin, a protein to which calcium is bound. The biologic effect of calcium is determined by the amount of ionized calcium, rather than the total calcium. The skeleton acts as a major mineral storage site for the element and releases Ca^{2+} ions into the bloodstream under controlled conditions. In mammals, levels of intracellular calcium are regulated by transport proteins that remove it from the cell. Ca^{2+} entering the cell plasma causes the specific action of the cell, whatever this action is: secretory cells release vesicles with their secretion, muscle cells contract, synapses release synaptic vesicles and go into processes of synaptic plasticity, etc. Ca^{2+} ions are one of the most widespread second messengers used in signal transduction. They make their entrance into the cytoplasm either from outside the cell through the cell membrane via calcium channels (such as Ca-binding proteins), or from some internal calcium storages. So, calcium metabolism or calcium homeostasis is the mechanism by which the body maintains adequate calcium levels. However, derangements of this mechanism can lead to "calcium-deficiency", which can have important consequences in health of "schizophrenic individuals". This concept is based on the demonstration that „NMDA receptor hypofunction“ can be based on calcium-deficiency, potentiated by nutritional hypoproteinemia (see Fig.2).

Vitamin D deficiency (calcium deficiency?) and schizophrenia

Evidence is accumulating to support the theory that vitamin D deficiency during pregnancy, caused by a lack of sunlight, can alter the development of a child's brain in the womb. The data for a link with schizophrenia is still controversial, but potentially worrying because vitamin D deficiency is so common. Vitamin D's role in building healthy brains had been largely ignored, until researchers began to spot some curious epidemiological trends. People who develop schizophrenia in Europe and North America are more likely to be born in the spring. And they are roughly four times as likely to be born to Afro-Caribbean immigrants living in England as they are to have parents of other ethnic origins living in the same areas.

The body needs sunlight to make vitamin D, and people with darker skin need more than paler-skinned people. So such observations led John McGrath of the Queensland Centre for Schizophrenia Research in Wacol to propose that a lack of vitamin D during early development tips the balance towards schizophrenia in genetically susceptible people (New Scientist, 21 July 2001).

Fig.2 Calcium deficiency may be a predisposing or causative factor in schizophrenia



What is necessary about the prevention of „NMDAR hypofunction“ ?

Activation of the NMDA receptor (NMDAR) channel can occur only if there is **simultaneous glutamate and glycine binding and partial depolarisation** of the membrane potential according to following steps (see NMDA receptors activation);

1. Sustained activation of AMPA receptors (AMPA) by, for instance, a train of impulses arriving at a pre-synaptic terminal, depolarises the post-synaptic cell, releasing the channel inhibition and thus **allowing NMDAR activation**

2. Ambient glutamate in the nerve synaptic cleft activates Ca^{2+} -permeable AMPAR on the postsynaptic neurons. This allows an **influx of Ca^{2+} that causes a gradual increase in intracellular calcium concentrations $[\text{Ca}^{2+}]_i$**

3. The recruitment of NMDAR during high presynaptic glutamatergic activity results in the permanent increase in synaptic efficacy known as **long-term potentiation (LTP)**. Influx of **Ca²⁺ through the NMDAR during LTP causes** the recruitment of AMPAR from intracellular stores to the synapse.

4. **Activation of AMPA presynaptic receptors** at physiological pH (pH 7.4) elicits the release of the [³H]transmitters in an external Ca²⁺-dependent manner (**so, AMPAR activation induces a Ca²⁺-dependent release** of noradrenaline, dopamine...)

5. **Activation of AMPAR permits activation of NMDAR** in the presence of physiological concentrations of Mg²⁺ (nerve endings are endowed with presynaptic receptors of the AMPA type, whose activation induces a Ca²⁺-dependent, exocytotic-like release of neurotransmitters).

6. **So, NMDAR activation begins** when sustained activation of AMPAR, **depolarises the post-synaptic cell**, releasing the channel inhibition and thus **allowing NMDAR activation**.

7. NMDAR activation occurs **only when the neuron is partially depolarized** (more positively charged), following activation of AMPAR

8. **NMDAR channel is blocked by Mg²⁺, which is removed upon depolarization**. So when glutamate and glycine bind and the cell is depolarized to remove Mg²⁺ block, the **NMDAR channel opens with consequent influx of Ca²⁺ and Na⁺** into the cell

9. On the other hand; at **hyperpolarized membrane potentials more negative than about -70 mV**, the concentration of Mg²⁺ in the extracellular fluid of the brain is sufficient to virtually abolish ion flux through NMDAR channels even in the presence of the coagonists glutamate and glycine

10. Astrocytes (glial cells) are important in controlling glutamate homeostasis. **[Ca²⁺]_i elevations in astrocytes stimulate the release of glutamate**, which acting on presynaptic or postsynaptic receptors, modulates synaptic transmission and neuronal excitability. So, astrocytic glutamate release is **Ca²⁺ -dependent** and can be triggered by **any ligand that stimulates an increase in [Ca²⁺]_i**

11. In addition, about simultaneous glutamate and glycine binding; **D-serine, SR enzyme and transporter GLYT-1; are present mainly in glial cells**

12. **D-Serine is the endogenous ligand for the glycine site** of the glutamate NMDAR, which coactivates postsynaptic NMDA receptors together with glutamate

13. The availability of D-serine depends upon the activities of **serine racemase (SR)** enzyme because SR directly converts L-serine to D-serine. **D-serine and SR are concentrated in astrocytes adjacent to NMDA receptors**, but less common or nonexistent in other neural tissues

14. Divalent cations such as **calcium or manganese are necessary for complete SR enzyme activity**. It was suggested that there might be a glutamatergic-mediated **regulation of SR activity by intracellular Ca²⁺ concentration**.

15. Glycine acts as an obligatory coagonist of glutamate at NMDAR. This role **depends on extracellular glycine levels, regulated by Na⁺/Cl⁻-dependent transporter GLYT-1**, present mainly in glial cells. High affinity transport by GLYT-1 is **regulated by [Ca²⁺]_i from intracellular stores**.

So, both SR and GLYT-1, as well as the glutamate transporters that protect against excitotoxicity, are expressed exclusively in astrocytes, indicating a **vital role of calcium-astroglia in modulating glutamatergic neurotransmission**.

NMDA receptors on the GABAergic interneurons of brain areas affected by schizophrenia are „NMDA hypofunctional“. This **hypoglutamatergic condition may induce a phenotypic loss of cortical parvalbumin- positive GABAergic interneurons**. There are reduced glutamatergic inputs onto the fast-spiking GABA cells that contain the **calcium-binding protein parvalbumin (PV)**. Given that PV interneurons express calcium-permeable AMPA receptors (which contribute to synaptic calcium signals), there was suggested that NMDA receptors exert a **tight control of intracellular calcium concentrations in these interneurons** and that decreases below a certain threshold induce the loss of their GABAergic phenotype.

Results suggest that the activity of NR2A-containing NMDA receptors play a pivotal role in the maintenance of the GABAergic function of parvalbumin (PV) interneurons. Calcium homeostasis may be particularly important in parvalbumin PV interneurons, and **calcium entering through NMDA and calcium-permeable AMPA receptors may play a fundamental role in the synaptic activation of these interneurons**.