Hypotheses and facts about BSE/ vCJD in the United Kingdom and the origins of the neurodegenerative diseases (31st World Veterinary Congress in Prague; September 17-20, 2013)

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1. HYPOTHESES AND FACTS ABOUT BSE/VCJD IN THE UK, SO MEAT AND BONE MEAL (MBM) AS INFECTIOUS AGENS SHOULD BE REVISED

Presented as the poster No 131;

Abstract

In Britain, perennial ryegrass mostly fed (1980s); protein content reached to over 300 g /kg dry matter in young, heavily with nitrogen fertilized grasses (high protein, potassium content); conditions for chronic hypomagnesemia (see; England - Wales; highest BSE incidence; rainy, cool weather and ryegrass - low magnesium content- feeding in high producing dairy cows, exception in the world).

In 1987/88; there 156 confirmed BSE cases in 145 cattle herds (with at least one confirmed case) and hypothetically was concluded, that BSE has an origin in MBM feeding, when majority of cows become infected as the newborn calves, based on the computer simulation models (see; only 1-2 cases BSE/ herd- not classical infectious epidemy; without experimental confirmation- MBM in calves feeding).

In 1991/92; there 513 dairy and 1266 suckler cow herds were sampled, and serum blood Mg below 0.8 mmol/l was found in 28% of cows. Later, 1993/94, there significantly higher dietary Mg- supplementation was realized and BSE incidence significantly decreased (Note; Czechoslovak patent -1991;

recommendation to increase the magnesium content by 180%, in feed supplements for cows).

Eurostat data (1990-2000) indicate that UK exports of MBM was in total; 229,000 tonnes, mostly to the Africa- Asia; however, not to Japan (see; after two decades later; in Japan 36 cases and Third World- no case of BSE, to date).

Dietary experiment (England, 2000) confirmed BSE (13% of 47 dairy cows), after long-term protein surplus, with ryegrass feeding (without MBM).

Introduction

Bovine spongiform encephalopathy (BSE) or "mad cow disease" still raises among people fears that humans can infect (new variant CJD) from cows by; (a) tainted meat, (b) infectious medical equipment- surgical instruments…,(c) infectious blood… These three important hypotheses is necessary to complete, and to add the base important hypothesis, known from 1988 (between the general public still recognized as valid!), that BSE in the United Kingdom (UK) was created after infection, when cows were fed meat and bone meal (WILESMITH et al. 1988). It was later supported by these two "phenomenons"; (a) ban on the feeding of meat and bone meal (MBM) to ruminants in the UK (1988) has resulted in a significant decline (1993) in the number of reported BSE cases (b) new variant form of Creutzfeldt-Jakob disease in humans (vCJD) has been discovered (1996), which may have been caused by BSE agens. However, there are several theories as to the origin of BSE. A common one is that scrapie crossed the species barrier through feeding MBM made from sheep infected with scrapie. The disease was then spread, through the UK cattle population, by feeding MBM to cattle from infected ruminant sources including meal made from cattle infected with BSE. Another mainstream theory of the origin of BSE is that it arose spontaneously in cattle, much as sporadic Creutzfeldt Jakob disease is believed to arise in humans.

BSE lesions are characterized by sponge-like changes in the brain seen under an ordinary microscope. Eating contaminated meat or other products from cattle (excluding dairy products) with BSE is thought to be the cause of vCJD. BSE is passed between cows through the practice of recycling bovine carcasses for MBM protein, which is fed back to other cattle. Both BSE and vCJD are fatal. Symptoms of vCJD involve psychiatric symptoms and behavioral changes, movement deficits, memory disturbances, and cognitive impairments.

European countries; about the highest incidence of BSE

	to 2012 BSE cases	to 2000 BSE cases	"BSE peak" year/ cases
UK	184 621	180845	1992/ 37 280
Ireland	1653	442	2002/ 333
Portugal	1082	159	1999/ 159
France	1015	80	2001/ 274
Spain	785	0	2003/ 167
Switzerland	464	335	1995/ 68
Germany	412	6	2001/ 125
Italy	144	2	2001/ 48
Belgium	133	19	2001/ 46
Netherlands	80	8	2002/ 24
Poland	73	0	2005/ 19
Czech Rep.	30	0	2005/ 8

Through the end of 2012; 184,621 cases of BSE (mostly to 2000 year; 180,845 cases) had been confirmed in the United Kingdom (UK) in more than 35,000 herds, BSE peaked in 1992 (37,280 cases).

In 2001, the EU introduced compulsory testing on BSE. As the table shows, until then was greater incidence of BSE detected only in Ireland, Switzerland, Portugal, France. Conversely, from 2001 there is a beginning of BSE incidence in other EU countries, see in particular Spain.

As in the UK after finding BSE in 1986 (in 1987, 446 cases) and similarly in Spain after finding BSE (2001, 82 cases); so in the previous period the population probably has consumed hundreds (thousands?) of BSE affected animals (including nerve tissue). However, after almost 30 years later, the resulting infectious new variant CJD (vCJD) in the UK almost not occurred (2007- 2011; only 18 cases), when in 2000 there this disease peaked (28 cases). How can this "phenomenon" be explained? In addition, the vCJD statistics are available (internet) only to 2011.

NOTE; The incidence of vCJD disease was (UK) from 1995 following; 1995 (3 cases vCJD);1996 (10);1997(10);1998 (18);1999 (15); 2000 (28); 2001 (20); 2002 (17); 2003 (18); 2004 (9); 2005 (5), 2006 (5); 2007 (5); 2008 (2); 2009 (3); 2010 (3); 2011 (5 cases vCJD); TOTAL (176 cases of vCJD). In the all world

media, there is **published number of 176 cases, in fact it was only 122 confirmed cases.**

The table also shows that in addition to UK – Ireland; also in Switzerland in the mid-90th years, has been the high incidence of BSE. And not only to 2000 but also later (from 2001; mandatory testing of BSE in EU) was a higher incidence of BSE in Western Europe, compared with Poland and the Czech Republic (where the lower milk yield in dairy cows). **In addition; were also cows in Switzerland fed infected MBM originating from the UK?**

Dairy cows with high milk production need in the feed ration a high concentration of protein. From experiences in the Czech Republic is known that almost all the 30 cases of mad cow disease (BSE) had been detected in high commercial dairy farms. For this, two cases of BSE have been repeatedly found in two herds of dairy cows. Even the last case was detected in the farm where high yielding dairy cows were fed organically (grass pasture) but had the **possibility of unlimited (ad libitum) intake of protein concentrate**. Why the BSE disease occurred only in highly commercial farms and not in the thousands of other breeds with a lower milk yield, when MBM was in the Czech Republic never fed in dairy cows (cattle)?

HISTORY about BSE/vCJD research; as a literature review

Why the BSE epidemic occured in the UK, especially?

1. Rainfall and the available water capacity of the soil were major forage yield determinants, with output in the UK ranging from 6000-14000 kg DM/ha under intensive fertilization in 1980s (LEE, 1988).
2. In Britain perennial ryegrass is the most important species of sown pastures; for example, the crude protein (CP) may range from as little as 30 g/ kg in very mature herbage to over 300 g/kg in young, heavily- fertilized grass (McDONALD et al., 1988).
3. Highest pasture yields in Europe, with a comparatively high share of grassland in total ruminant feed composition- 83% (LEE, 1988).
4. According to "50 – year review" about the fertilizer applications in the UK (HEMINGWAY, 1999), there was mayby highest nitrogen fertilizers consumption in the world; in England and Wales, especially (1983-1988).
5. In Britain- Ireland, a high intake of grasses in ruminants; available water capacity, high N (and K-fertilization by animal excrements), cool and cold

marine climatic region; these circumstances are ideal for the subclinical (chronic) hypomagnesaemia in ruminants.

6. However, in the UK, there was not interest about the agronomic Mg-research in ruminants within twenty years period (1976- 1996). In contrast it can be interpreted, acording to the study of HEMINGWAY (1999); that to the mid of 1970s, in the UK there the agronomic Mg research was greatest in the world.
7. Also, according to the DUA and CARE (1995), there was the greatest "veterinary" Mg-research in ruminants in the world; unfortunately only to the mid of 1980s.
8. However, with the high probability that after significant increase of crude protein in dairy rations (ARC, 1980) in mid- 1980s, it was without equality of oral Mg-supplementation.
9. This can show on the real probability about the "abnormal" Mg-deficit in British ruminants from 1950s to 1980s. From both publications, there is evidence about reality of the Mg-deficit also in the next decade years (1985-1995) in British ruminants.
10. In 1991/92; there 513 dairy and 1266 suckler cow herds were sampled, and serum blood Mg below 0.8 mmol/l was found in 28% of cows (McCOY et al., 1993). And after 1993/94 period, there is some evidence about the increase of additional dietary Mg-supplementation in the UK dairy rations (McCOY et al., 1994).

1988; First epidemiological BSE study suggested that the majority of cows become infected by MBM in calfhood

First epidemiological study suggested (see; "suggested" only- not "found") that the majority of cows become infected by BSE (MBM feeding), when they are calves, and that the disease may be a variation of scrapie – a disease in sheep, which has altered and ‘jumped’ species. Already this **initially a computer simulation model (WILESMITH et al., 1988), was** constructed in intention determine the hypothesis that BSE is caused by a transmissible agent.

Summary; This study, initiated in June 1987, describes the epidemiology of bovine spongiform encephalopathy (BSE), a recently described novel neurological disease of domestic cattle first identified in Great Britain in November 1986. Records suggested that the earliest suspected cases occurred in April 1985. There was variability in the presenting signs and the disease course, but the majority of cases developed behavioural disorders, gait ataxia, paresis and loss of bodyweight; pruritus was not a predominant sign. The form of the epidemic was typical of an extended common source in which all affected

animals were index cases. The use of therapeutic or agricultural chemicals on affected farms presented no common factors. Specific genetic analyses eliminated BSE from being exclusively determined by simple mendelian inheritance. Neither was there any evidence that it was introduced into Great Britain by imported cattle or semen. The study supports previous evidence of aetiological similarities between BSE and scrapie of sheep. The findings were consistent with exposure of cattle to a scrapie-like agent, via cattle feedstuffs containing ruminant-derived protein. It is suggested that exposure began in 1981/82 and that the majority of affected animals became infected in calfhood.

Results; The frequency of the presenting clinical recorded for 156 confirmed cases; The most common history given by the herdsmen was "nervousness". The distribution of 145 herds, with at least one confirmed case, by their cattle purchasing policy and the presence of sheep on the farm since 1980 showed that in 15 per cent of herds no cattle had been purchased and 20 per cent of herds had had no contact with sheep. **The use of a computer based simulation model** indicated that the values of the age specific incidences observed in 1987 were consistent with the following features. First, both calves and adults (over two years old) have been exposed, but the risk for calves was 30 times that for adults; secondly, exposure of the cattle population commenced in the winter of 1981/1982 and continued to at least the end of 1984 and, thirdly, an incubation period with the range 2-5 years to at least eight years and a log normal distribution. The maximum incubation period that could have been observed in 1987 was six years. Further epidemiological data for 1988 and subsequent years is needed to determine whether exposure continued after 1984.

NOTE; In the text of article "Bovine spongiform encephalopathy: epidemiological studies" (WILESMITH et al. 1988), which became the basis for infectious "BSE – scrapie – vCJD" hypotheses, there no information about MBM feeding. So, **multiple cases of the BSE were found (145 cattle herds), when no MBM was fed to ruminants, in the field conditions!** No experiments have been conducted if cattle were fed MBM, infected with BSE under controlled conditions to show, unequivocally, that MBM can act as the vector of BSE.

1989; Epidemiology of BSE in Northern Ireland and newborn calves feeding

At the same time (since 1988), in N.Ireland – no feeding MBM in cows; WILESMITH´s team, four years later published; "Bovine spongiform encephalopathy in Northern Ireland: epidemiological observations 1988-1990" (DENNY et al. 1992).

Later, by detailed epidemiological investigation of BSE (at the same time, since 1988 - 1680 cases), WILESMITHˇs colleagues found also "no feeding MBM" in cows (only "suggestion"). See the article "Epidemiology of bovine spongiform encephalopathy in Northern Ireland 1988 to 1995" (DENNY and HUESTON, 1997).

Perhaps infections in calves as a "computer model findings", were based on the authors (WILESMITH et al., 1988) "personal" experience from one dairy herd (Northern Ireland), published (April 1989) in the " British Veterinary Journal" (WINTER et al., 1989).

And about results, writing in this article? **Fourteen cases of bovine spongiform encephalopathy (BSE) were diagnosed on the basis of clinical examination in a closed herd of British Friesian cows during a 9-month period** from October 1987 until June 1988. No protein of animal origin had been fed to either heifers or cows in this herd during the past 5 years and there had been no direct contact with sheep. The epidemiology of BSE in this report suggests that, if the postulate of Morgan (1988) is correct, infection is ingested within the first 6 months of life and there then follows a 4-5-year period before clinical signs appear".

The herd consisted of 500 cows, the average lactation yield was 5500 litres in 305 days. Following calving, the cows were fed grass silage on a self-feed basis from the clamp face. Since 1983 only maize gluten has been added to the ration fed at a flat rate of 5 – 0 kg per head per day. However, only in the chapter "Discussion" (not as results) was commented, see the text; "Only 12 kg of animal "protein of ruminant origin" had been incorporated into the proprietary compound feedstuff fed to the calves from 2 weeks old to 6 months old". Additionally, it is interesting that in this scientific journal is a suspected infection written in the study summary, which is based only on brief and inaccurate data on feeding animal protein (**meat meal or MBM or another ruminant protein supplement?**), to calves, mentioned in the discussion of the study. More important, however, **inaccurate data (maize gluten meal or maize gluten feed?)** in the results of a study which says that the cows were fed 5 kg of corn gluten. If it was a maize (corn) gluten meal and daily dose of 5 kg, it would represent a daily intake of 2.5 kg of protein, which would mean a huge excess of protein. Corn gluten feed should not be confused with corn gluten meal. Corn gluten meal has 2 times the protein content (50%) of corn gluten feed (25%).

And what was published about animal protein fed to calves? In this time of the mid of 1980s WARNER (1984) reported no advantage to using undegradable protein (UDP) sources in calf starter diets! Twenty years later

(2004), see the study "Calf Starter Research, Protein Sources for Calf Starters"; there was concluded that; Sources of rumen undegraded protein appear to have little value for starters. Also, there appears to be no advantage to feed starters that contain more than 18% crude protein, even when the starters are formulated to be equal in rumen degradable and metabolizable protein.

Higher additional dietary Mg-supplementation in dairy cows as an European "phenomenon" at the beginning of 1990s; and decrease of BSE incidence in the UK

The official scientific statement about the 5-years incubation period of the BSE is based on the feed ban of MBM (1988) and the BSE incidence decrease (after 1993). Putting this"phenomenon" into the practical conditions; significantly higher additional dietary Mg-supplementation - can be a cause about the BSE incidence decrease in the UK, after 1993/94 period.

See the article; "Providement for an optimum supply of sodium and magnesium to the feed rations of dairy cows and high pregnant heifers" (HLASNY, May 1989) published in Czechoslovak scientific journal "Biol. Chem. Vet." (Prague). These results were also published by the"Czechoslovak Patent Office" in Prague (HLASNY, 1991), see "Mineral supplement for breeding cattle". Patent No. 274 171. These recommendations that much more magnesium (by 180%) is necessary in dairy mineral supplements; really, it was commonly realized in Europe, at the beginning of 1990s.

This phenomenon about the "European great Mg interest" at the beginning of 1990s, we presented at the "3rd European Congress on Magnesium" (March, 1990) in Geneve, see the article; "Mechanism of the origin of magnesium deficiency in feedstuffs and in nutrients", published in "Magnesium Research" (HLASNY and STEIDL, 1990).

Increasing interest about dietary cattle Mg-supplying in the UK from 1993/94

In the UK veterinary journals (period; 1985-1995), there is only one information (article) about the cow hypomagnesaemia testing (McCOY et al., 1993)- from Northern Ireland (clotted blood samples submitted under the Brucellosis Eradication Scheme were used for this survey). There, 513 dairy and 1266 suckler cow herds were sampled during the grazing season from March to November 1991 (to February 1992- suckler cows). It was found; serum blood Mg below 0.8 mmol/l in 14.1 of dairy and in 33.9% of suckler cows. The peak of hypomagnesaemia incidence; in both dairy and suckler herds occurred in the

period from March to June. In addition, in 8.2% of suckler cows – the blood Mg below 0.6 mmol/l was found!

One year later, report from McCOY et al. (1994) describes a novel method to evaluate the most popular commercially available hardened magnesium blocks – as oral mineral Mg-supplement in cattle feeding. There are other informations (from their survey- article); about the evidence of Mg- oral supplementation increase in the UK cattle. There is evidence that in 1992/93 period; additional dietary magnesium- preferably be given as an oral mineral supplement, rather than by attempting to modify the mineral content of herbage- by Mg fertilization.

1999; Agronomic "Mg- research" in the UK, findings

HEMINGWAY, R.G.; The effect of changing patterns of fertilizer applications on the **major mineral composition of herbage in relation to the requirements of cattle: a 50 – year review**. Animal Science, 69, 1999; 1-18

According to the paper of HEMINGWAY (1999), in the **UK there was the greatest "agronomic" Mg-research (in ruminants) in the world; unfortunately only about to the mid- 1970s.** This great Mg-research shows on the real probability about the "abnormal" Mg-deficit in the UK ruminants from 1950s to mid-1980s. However, later (1976- 1996) in the UK, there was not interest about the Mg-research. In addition, within this 20-years period, there is the begining of the BSE incidence in the UK.

This article shows that the long time research about the NPK fertilization in the UK has been sumarized. In addition, the results based on the literature review; from the long time of high experienced author are important **about the "estimate" of Mg-deficit in the UK ruminants.** There is evidence, that in the UK was the intention to use high rates of N-fertilizers, especially; therefore hyperammonemia in ruminants can be involved.

In addition, it seems that there is the similarity between the individual susceptibility in the hypomagnesaemic "grass tetany" and the BSE ("indicator cows" only); because the incidence of the BSE within a dairy herd of similar animals was very low- from 0.14 to 3.3% (WILESMITH et al., 1988).

1996 - 2009; The discovery of the human form of BSE, new variant CJD (vCJD)

In 1995 and early 1996, a small number of cases of CJD with a remarkably early age at death (29 years) were identified in the UK, with an unusual clinical and pathological phenotype for CJD, including extensive deposition in the brain of

florid plaques. Research of this discovery was initiated by Dr. RG WILL (National Creutzfeldt-Jakob Disease Surveillance Unit in Edinburgh), that wrote the letter to UK neurologists (March 21, 1996), there among other things he wrote the following;

"In the past few weeks we believe we may have identified a new clinico-pathological phenotype of CJD which may be unique to the United Kingdom… **COULD YOU PLEASE NOTIFY THE CJD SURVEILLANCE UNIT OF ANY SUCH CASE WITH THIS CLINICAL OR NEUROPATHOLOGICAL PROFILE, WHETHER OR NOT THE PRESENTATION IS IN THE YOUNGER AGE GROUP. COULD YOU ALSO CHECK YOUR RECORDS AND NOTIFY ANY SIMILAR CASES THAT MAY IN RETROSPECT FIT WITH THIS CLINICOPATHOLOGICAL PROFILE**. Four of the recently identified cases were confirmed by brain biopsy. If you are considering brain biopsy in any suspect cases of CJD it is essential to follow the Department of Health guidelines which state that neurosurgical instruments used on any case of CJD must be destroyed and not reused… The identification of a form of CJD that might be casually linked to BSE will result in widespread anxiety and concern..."

After sending this letter to neurologists, **very quickly (15 days later) was the discovery of new disease published (Will RG at al, Lancet. 1996 Apr 6)**, there scientists say the following (Findings and Interpretation);

"Ten cases of CJD have been identified in the UK in recent months with a new neuropathological profile. Other consistent features that are unusual include the young age of the cases, clinical findings, and the absence of the electroencephalogram features typical for CJD. Similar cases have not been identified in other countries in the European surveillance system. These cases appear to represent a new variant of CJD, which may be unique to the UK. This raises the possibility that they are causally linked to BSE. Although this may be the most plausible explanation for this cluster of cases, a link with BSE cannot be confirmed on the basis of this evidence alone. It is essential to obtain further information on the current and past clinical and neuropathological profiles of CJD in the UK and elsewhere".

However, sporadic CJD disease has been diagnosed previously also in young patients (PACKER et al. 1980; BROWN, P et al. 1985; BRITTON et al. 1995; BATEMAN et al. 1995), but these are usually isolated case reports and there was no evidence of PrP plaques. However, BROWN P. et al (1985) pointed out that a histopathologically-verified, clinically typical case of Creutzfeldt-Jakob

disease (CJD) was described in a 19 year-old girl and 3 previous cases of CJD have been reported in adolescents.

Panic ensued when it was discovered that humans that ate infected cattle meat could develop an equally horrific variant of the vCJD disease, after WILL RG et al, vCJD discovery in Lancet (April 1996). **Immediately afterwards, in May 1996, not only in Britain but throughout the world began to spread the news of the vicious and incurable disease transmitted from animals to humans.**

Next supporting evidence (gradually to 2009) about BSE and vCJD transmission is mostly related to description in **high-ranking journals** (Lancet, Nature, Science, PNAS…), See some articles about **to obtain "further informations";**

1. 'New variant' Creutzfeldt-Jakob disease (CJD) has strain characteristics…, consistent with BSE being the source of this new disease. **COLLINGE J et al.. Nature. 1996 Oct 24**
2. We now find that the biological and molecular transmission characteristics of vCJD are consistent with it being the human counterpart of BSE**. Hill AF et al. Nature. 1997 Oct 2**
3. Our data provide strong evidence that the same agent strain is involved in both BSE and vCJD. **Bruce ME et al., Nature. 1997 Oct 2**
4. There is now considerable concern that bovine prions (BSE) may have been passed to humans, resulting in a new form of CJD. **PRUSINER SB; Science. Oct. 10, 1997**
5. Current evidence strongly supports the hypothesis that there is a causal link between BSE and nCJD disease. **WILL RG, Dev Biol Stand. 1998**
6. Prions are unprecedented infectious pathogens that cause a group of invariably fatal neurodegenerative diseases by an entirely novel mechanism. **PRUSINER SB., Proc Natl Acad Sci U S A. 1998 Nov 10**
7. Our findings provide the most compelling evidence to date that prions from cattle with BSE have infected humans and caused fatal neurodegeneration. **Scott MR et al., Proc Natl Acad Sci U S A. 1999 Dec 21**
8. As of December 31, 1998, 35 deaths had been attributed to new variant Creutzfeldt-Jakob disease (nvCJD) in the United Kingdom. The median illness duration was 14 months (range, 8-38 months) and the median age at death was 29 years (range, 18-53 years). **WILL RG et al. Ann Neurol. 2000 May.**
9. Our findings raise the possibility that this infection was transfusion transmitted. Infection in the recipient could have been due to past dietary exposure to the BSE agent. However, the age of the patient was well beyond that of most vCJD cases. **Llewelyn CA, et al. Lancet. 2004 Feb.**

10. Commentary: The risk of vCJD Disease: reassurance and uncertainty. WILL RG, Int J Epidemiol. 2005 Feb.
11. Identification of possible transmission of vCJD via blood transfusion has caused concern over spread of the disease within the human population. **Bishop MT et al. Lancet Neurol. 2006 May**
12. Incubation periods of prion infection in humans can exceed 50 years. **Wadsworth JD and COLLINGE J.; Biochim Biophys Acta. 2007 Jun**
13. Bovine imports from the UK may have been an important source of human exposure to BSE and may have contributed to the global risk for disease. **Sanchez-Juan P et al., Emerg Infect Dis. 2007 Aug**
14. Recent advances suggest that prions themselves are not directly neurotoxic, but rather their propagation involves production of toxic species, which may be uncoupled from infectivity**. COLLINGE J and Clarke AR., Science. 2007**
15. Our data presented here in two murine models suggest no significant alterations to transmission efficiency of the agent following human-to-human transmission of vCJD. **Bishop MT et al, PLoS One. 2008 Aug 6**
16. A retrospective review of the medical case notes of the deceased recipients of vCJD- implicated blood components found no evidence that any further cases…. **Gillies M, et al. Vox Sang. 2009 Oct**

NOTE; The last two articles (2010) suggest that the entire scientific saga of BSE transmission to humans has questionable foundations, and that **both CJD diseases are not infectious, because they occur only sporadically.**

Variant or sporadic Creutzfeldt-Jakob disease? Brandel JP et al. Lancet. 2010 Mar 13; **Variant CJD: where has it gone, or has it?** Will B; Pract Neurol. 2010 Oct;

2000;The BSE disease was tested in dairy cows;"nutritonal experiment" performed in England, see three articles;

1. Effects of altering energy and protein supply to dairy cows during the dry period. 1. **Intake, body condition, and milk production**. Dewhurst RJ, Moorby JM, Dhanoa MS, Evans RT, Fisher WJ.; J Dairy Sci. 2000 Aug
2. Effects of altering the energy and protein supply to dairy cows during the dry period. 2. **Metabolic and hormonal responses**. Moorby JM, Dewhurst RJ, Tweed JK, Dhanoa MS, Beck NF.; J Dairy Sci. 2000 Aug
3. **Aspects of the metabolism of dairy cows during the incubation of bovine spongiform encephalopathy**. Moorby JM, Dhanoa MS, Austin AR. Vet Rec. 2000 Oct

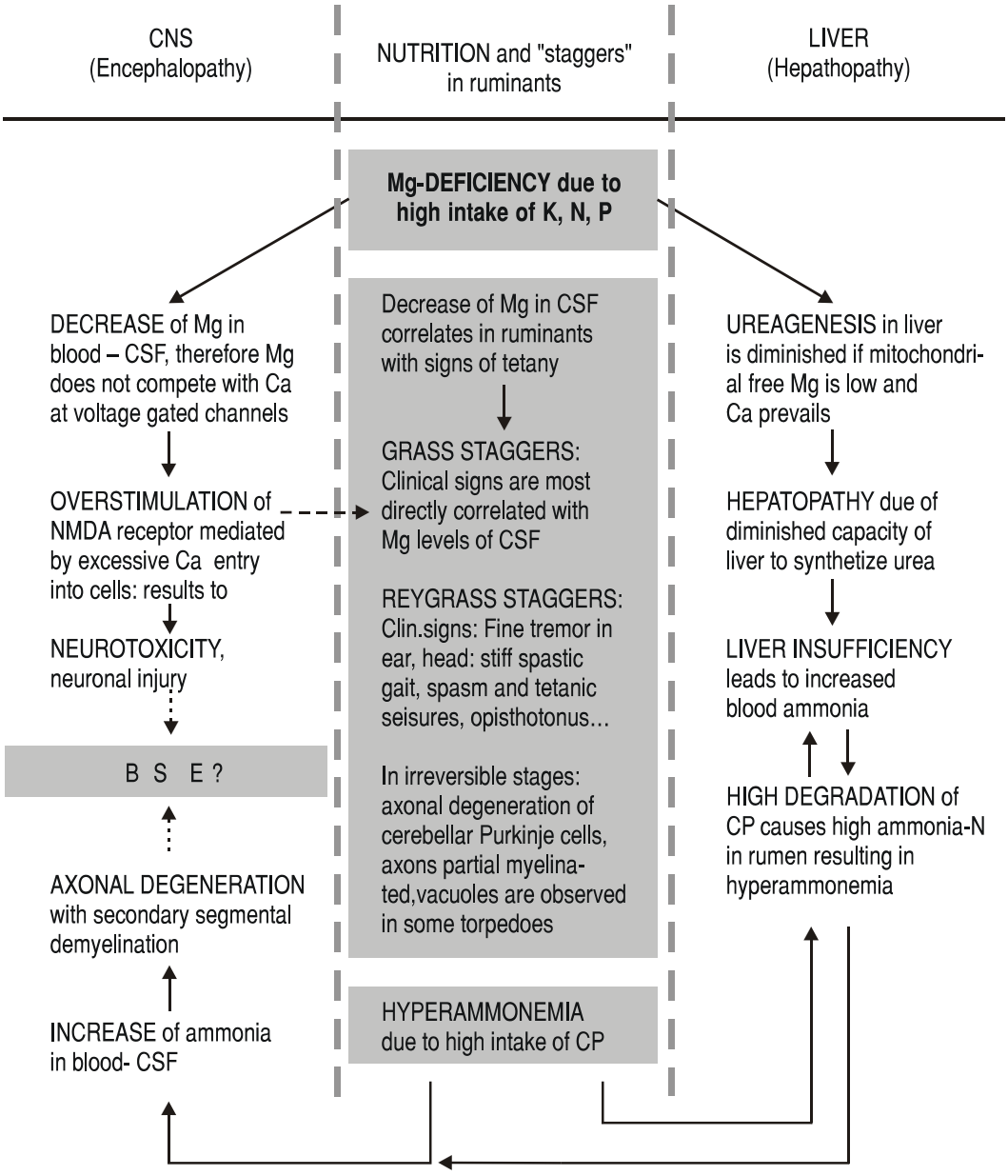
This experiment was conducted using diets and other conditions typical of northwestern Europe, under well defined conditions of husbandry and nutrition, without MBM feeding. The effect of altering the amount of protein and energy over the final 6 wk of the dry-period diet and during the first 21 wk of the subsequent lactation was investigated, in 47 dairy cows. Perennial ryegrass silage was used ad libitum; final 6 wk of the dry-period diet and during lactation plus a concentrate with high crude protein (CP) level (22.5%) was fed. During lactation daily total dry matter (DM) intake was ca 17.4 kg; the content of CP (N x 6.25) was ca 20% during first 12 weeks, and ca 17.5% of CP in the diet DM, to the 22 wk of the lactation period. So, very high CP concentrations in the diet used, and high levels of plasma urea-N (38- 43 mM) were found during lactation and also during dry period (30- 36 mM). No clinical metabolic disorders were recorded. However, after the collection of the last blood sample (21 wk of lactation), six of the 47 animals (so; 13 per cent!) developed clinical signs of BSE (later histopatologicaly confirmed). Although when they were sampled it was not known that they were incubating the BSE. So after **long-term (28 weeks) dietary protein surpluss (18- 20%) was fed, when only 15% of protein in feed ration was needed, and cca 13 percent of dairy cows developed BSE!** Such a high percentage of BSE disease was never under normal conditions in none of British cow herds found.

NOTE; Unfortunately, this very detailed nutrition experiment was not focused on the metabolism of magnesium. The results on this important trial, were nowhere and never refered after 2000 (as a reference to studies on BSE)!

When these articles about "ryegrass toxicity" on the CNS of dairy cows were published; it was prompted to air my views in studies of CNS neurotoxities by nutritional causes. I reviewed about 200 papers on the CNS changes associated with BSE, and detected a possibility that these mechanisms have a strong influence on CNS, especially in ruminants, and that the BSE has its roots in a more common nutritional problem (HLASNY, 2001), see figure;.

The mechanism of the influence of magnesium deficiency with protein excess; in the diet of ruminants (an example when ryegrass is fed) on the NMDA receptor and the emergence BSE (Hlasny, J.: Výzkum v chovu skotu; March 2001)

Nervous diseases and connections with nutrition in ruminants



CNS: Central nervous system • CSF: Cerebrospinal fluid • CP: Crude protein
NMDA: "N-Methyl-D-Aspartate" receptor.

This alternative "BSE ammonia- magnesium theory" is based on the chronic Mg-deficiency potentiated by hyperammonemia in ruminants. This theory was introduced with the well- known fact, that over the past 50 years yields (to 2000) of many crops have increased roughly in proportion to the increase in NPK–fertilizer application. Luxury consumption of potassium (K) fertilizers, leads to

distortion of cation ratios in the herbage: concentration of magnesium (Mg) was reduced relative to potassium, especially.

According to the literature sources, lush grass innately has an increased level of crude protein. As this protein is readily fermentable, it leads to increased intraruminal ammonia concentrations up to 30- 70 mmol/l (MARTENS and RAYSSIGUIER, 1980) and to a decrease in the availability and absorption of Mg (MARTENS et SCHWEIGEL, 2000; URDAZ et al., 2003; FONTENOT et al., 1989). It has been observed that application of nitrogen (N) fertilizer, which may increase potassium (K) uptake by plants and/or decrease Mg utilization by livestock, often is associated with the occurence of tetany (FONTENOT et al., 1973). The nutrient having the greatest adverse effect on Mg absorption is an excess of K in the ration, as shown by at least four sheep experiments (GRACE, et al, 1988; DALLEY, et al, 1997; WACIRAPAKORN, et al, 1996).

While non-ruminants absorb Mg primarily from the small intestine, ruminants are able to absorb much of their Mg requirement from the rumen. In fact, the reticulum and rumen can account for up to 80% of the Mg absorption along the entire digestive tract (REMOND et al. 1996). Another experiment with Holstein cows fed a "total mixed ration" (TMR) confirmed that the rumen was the major site of Mg absorption (KHORASANI et al. 1995). The underlying mechanisms of this impaired Mg^{2+} absorption from the rumen have been studied intensively by in vivo and in vitro methods (CARE et al., 1984; MARTENS et al., 1987), and there is a growing body of evidence that the active and transcellular component of net Mg^{2+} uptake is significantly decreased by high ruminal [K^{+}] (MARTENS et al., 1987). There are many other controlled feeding trials showing the negative relationship between K intake and Mg absorption (SMYTH et al., 1958: KEMP, 1960: NEWTON et al., 1972; POE et al., 1985; WYLIE et al., 1985). The highest potassium (K) level in a dairy cows TMR resulted in reduced plasma Mg and reduced milk yield (FISHER, et al, 1994; KHORASANI et al, 1997; FREDEEN, et al, 1995). In addition, the later study (JITTAKHOT et al., 2004) confirmed earlier works showing that the addition of K to the ration of ruminants inhibits Mg absorption.

Are common the clinical signs of BSE and magnesium deficiency in cows?

a) The clinical signs of BSE

Most cases of BSE in Great Britain have occurred in dairy cows between 3 and 6 years of age with the initial clinical sigs: nervous, kicking, locomotor difficulty, loss of condition, loss of weight, reduced milk yield, abnormal behaviour, nervous of entrances, temperament change, falling, aprehension,

aggression, dificulty rising, tremors, hyperaesthesia, and recumbency. Other clinical signs analysed for the 17,154 cases; ataxia, kicking in parlour, excessive licking, head pressing or rubbing, abnormal ear position, teeth grinding, abnormal head carriage, head shyness… There were variations in the frequency with which some signs were recorded in animals observed at different times during the epidemic (WILESMITH et al., 1992).

b) The causes and clinical signs of hypomagnesemia

Forages causing staggers in livestock include perennial ryegrass must be considered in the differential diagnosis of hypomagnesemia. Expression of clinical signs in "ryegrass staggers" is most directly correlated with the cerebrospinal fluid (CSF) Mg levels (SMITH, 1996). Several factors adversely influence Mg metabolism in cattle and may "trigger" grass tetany.: among them drastic fluctuations in spring temperatures, prolonged cloudy weather, organic acid content of plants, hormonal status of the animal, level of higher fatty acids in plants, energy intake of the animal, and additional stress – such as a dog chasing animals, parasites, or a cold rain.

Why hypomagnesemia is not observed in ruminants on warm season grasses?

There is the explanation; these grasses are low in crude protein and potassium, and higher in magnesium content; grown under a low NPK-fertilizers application (hot weather- water stress is obvious). The main advantages of the grasses are their summer growth habit, providing when temperate (cool) grasses (perennial ryegrass, orchardgrass…) have become semi- dormant, and their ability to grow to use soil moisture efficiently. By the same token, they share the disadvantage of all tropical (warm) or C_4 grasses in that their nutritive quality for livestock is lower than that of temperate (C_3) species. This appears to be related to higher fiber and lower crude protein, and potassium concentrations in the warm season grasses (REID and JUNG, 1982).

It is well established that tropical grasses contain relatively high concentration of fiber and low levels of protein (PAYNE, 1966; BUTTERWORTH, 1967). The fundamental differences in leaf structure (Kranz anatomy) and metabolism of C_4 grasses result in marked differences in composition and nutritional quality of tropical and temperate forages (NORTON, 1982). Environmental conditions exert a strong effect on composition of C_4 grasses result in slower rates of degradation of fiber components in rumen (AKIN, 1986), and lower digestibility by ruminants (MINSON, 1981).

Under tropical or subtropical conditions, pastures based on C_4 grasses are generally considered to provide no more than a maintenance level of nutrition

for grazing animals. There are clear differences in the concentration of minerals; lower levels of Ca and P, and higher concentrations of Mg, Cu in tropical than in temperate grasses (NORTON, 1982); and K concentrations quite low (mean 1.23% for 378 samples) with a high positive correlation with crude protein (REID and JUNG, 1988)in tropical grasses. Tropical grasses appear to contain higher concentrations of Mg (0.36%) than temperate grasses (0.18%) - according to NORTON (1982).

So neurodegenerative diseases, occurred to a greater extent, only in ruminants (BSE, scrapie), because only in them, magnesium is not absorbed in the intestine, but in the rumen. The excess of protein-nitrogen in the rumen decreases absorption of magnesium. Most suffer with magnesium deficiency, high yielding dairy cows, in which high milk production leads to the dysbalancy between calcium and magnesium. Prolonged magnesium deficiency leads to an excess of calcium in animal tissues, and NMDA receptor hyperfunction.

And a final question?

Why the low incidence of BSE in the U.S. when there is a significant incidence of "grass tetany" in cows?

Response: Because in the U.S. in nature only acute form of hypomagnesemic tetany is found. This is due to the fact that there are alternating periods of rain with the prevailing drought, so the "plateau" long-term high protein content in grasses are not present there. In contrast, in the Britain during all year round humid maritime climate, ideal conditions for the occurrence of chronic, subclinical hypomagnesemia.

The BSE Inquiry in the UK, October 2000

The BSE Inquiry was announced in Parliament on 22 December 1997, and set up on 12 January 1998, to establish and review the history of the emergence and identification of BSE and new variant CJD in the United Kingdom.

The cause of BSE; By the end of 1987 Mr John WILESMITH, had concluded that the cause of the reported cases of BSE was the consumption of MBM, which was made from animal carcasses and incorporated in cattle feed. The following provisional **conclusions of Mr WILESMITH, which were generally accepted** at the time as a basis for action, were reasonable but fallacious:

- the cases identified between 1986 and 1988 were index (ie, first generation) cases of BSE;
- the source of infection in the MBM was tissues derived from sheep infected with conventional scrapie;

- the MBM had become infectious because rendering methods which had previously inactivated the conventional scrapie agent had been changed.

However, The BSE Inguiry "Committee" said; The cases of BSE identified between 1986 and 1988 were not index cases, nor were they the result of the transmission of scrapie. They were the consequences of recycling of cattle infected with BSE itself. The BSE agent was spread in MBM.

BSE probably originated from a novel source early in the 1970s, possibly a cow or other animal that developed disease as a consequence of a gene mutation. The origin of the disease will probably never be known with certainty. The theory that BSE resulted from changes in rendering methods has no validity. Rendering methods have never been capable of completely inactivating TSEs.

Opinion on; Hypotheses on the origin and transmission of BSE in cattle, adopted by the Scientific Steering Committee at its meeting of 29-30 November 2001

With regard to the origin of BSE;

The origin of the BSE prion is not known. Many hypotheses have been suggested, including for example an origin from mammalian species other than cattle; a mutant form of scrapie agent, a natural TSE in Bovidae or Felidae or other wild animals whose carcasses were rendered into MBM, the existence of a form of sporadic TSEs like CJD of humans, a spontaneous mutation of normal bovine PrP into an infectious and protease resistant TSE prion etc. For none of these hypotheses is there enough data to either substantiate or to reject it.

Recommendations about lower protein intake in dairy cows as a "phenomenon" at the 2001/2002 period; and decrease of BSE incidence

At the beginning of 1980s, for more productive high yielding dairy cows more of protein was required, including UDP (fish meal, gluten meal, MBM...) incorporation, especially to lactating cow rations.

During early lactation (0-70 days postpartum) milk production increases rapidly, peaking at 4 to 6 weeks after calving. Protein content is critical during early lactation; rations may need to contain **19% of more crude protein (ENSMINGER et al., 1990)**, with 0.2% of Mg in dry matter (DM) dairy ration. Almost the same high protein recommendations (18.8 % of dry matter); McCULLOUGH (1994) recommended to dairy rations of high producing "supercows"- however, during the "all lactation" and with higher Mg (0,33%) in

DM dairy ration. On the other hand, according to **the NRC (1989)** this high protein level it was recommended **only during first three weeks (0-21 days postpartum) after calving**. The research during 1990s resulted to decrease of protein content in dairy rations; see the comparison of the NRC (1989 and 2001);

Dairy cow: 600-680 kg body weight				
	Lactation			Early lactation
Milk yield (kg/day)	35	45	55	35
Degradable protein – "DP" (%):				
NRC,1989	9,7	10,4	10,4	9,7
NRC,2001	9,7	9,8	9,8	10,3
Undegradable protein- "UDP" (%):				
NRC,1989	5,7	6,0	6,3	7,2
NRC,2001	5,5	6,2	6,9	5,6
Crude protein – "CP"- (%):				
NRC,1989	16,0	17,0	17,5	**19,0**
NRC,2001	15,2	16,0	16,7	**15,9**

Therefore if we will put into practice; the recommendation from the NRC (January 2001) about the significant decrease of crude protein in the early lactation - this "phenomenon" can be a cause about the BSE incidence decrease in the western Europe (UK, Ireland,France, Netherlads, Switzerland…) especially, after 2001/2002 period. There were high producing dairy cows in 1990s, compared with the eastern Europe countries..

New norm (NRC 2001), apparently got **into practice in Poland, the Czech later** (see; there BSE peaked in 2005) than in Western Europe. For example, in the Czech Republic in 2002 (November) were in the journal "Farmer" in the article "Urea and cow health"; **highlighted benefits of high protein intake** in the diet of high producing dairy cows, unfortunately, by "older U.S. methods" (McCULLOUGH, 1994).

2004- 2006; Four cases about the "convincing evidence" that vCJD infections have been transmitted by blood transfusion

Shortly after vCJD was recognized (1996), authorities in many countries became concerned about the possibility that it could be transmitted from one person to another through contaminated blood supplies used for transfusion in hospitals. It was in 2000- 2002 supported by sheep experiments, when transmission of BSE has been repeatedly achieved by blood transfusions (HOUSTON et al. 2000; HUNTER and HOUSTON, 2002).Already a few months after these findings, efforts followed, again with the participation of professor R.G.WILL, and convince other scientists, that disease transmission by blood is also possible in humans. Again, (as vCJD) it was very quickly published in February 2004 (also in Lancet), see a case report; In December 2003, the first likely transmission of variant CJD by blood transfusion was described, lead by the team of WILL RG (LLEWELYN et al. 2004). **The victim developed symptoms of vCJD 6.5 years after receiving a blood transfusion at the age of 62!** In response to this case, the UK government announced a new policy excluding those who received blood transfusions after 1980 from donating blood. Quickly, in August 2004, researchers published (PEDEN et al 2004) another 2 nd worrisome case, also in Lancet. In July 2004, a second recipient of non-leukoreduced red blood cell concentrate from another such donor in the U.K. was reported to have died of other causes without clinical or neuropathological evidence of vCJD, but at autopsy the recipient had abnormal accumulations of prion protein in lymphoid tissues (PEDEN et al 2004). However, second and other cases of vCJD disease transmission (see references in the world media), are confusing, and it is difficult to determine if three or four cases of blood transmission were found in total. Two additional recipients of non-leukoreduced red blood cell concentrates from a donor incubating vCJD were subsequently reported by U.K. authorities in February 2006 and January 2007 have died with confirmed vCJD (HEWITT et al. 2006). These four cases provided convincing epidemiological evidence that vCJD infections have been transmitted by non-leukoreduced red blood cell concentrates…

2006; Is prion protein really infectious or is merely a secondary marker of the presence of the scrapie agent?

To determine the mechanisms of intestinal transport of infection, and early pathogenesis, of sheep scrapie, isolated gut-loops were inoculated (JEFFREY et al., 2006). Firstly, it is known that individual people and animals have different

levels of genetic susceptibility to this group of diseases, but no one knows how this resistance is achieved. One option is that resistant people do not absorb the disease-associated prion protein (PrP) from their guts. To test this, the researchers worked with 50 sheep, with different degrees of genetic resistance to scrapie – the sheep form of the disease. When they injected material containing abnormal prion protein (PrP) into the sheep's gut, it was equally absorbed by all sheep. "This clearly shows that resistance is not achieved by blocking uptake of abnormal proteins from the gut – it must be achieved by some other mechanism," says lead author Dr Martin Jeffrey.

Secondly, they looked in more detail at the route of absorption in the gut. Using surgically modified sheep, they loaded a small area of the gut with a fluid mixture containing 0.5 grams of scrapie infected brain containing a large amount of the disease specific variant of the PrP protein and watched how it was taken up. Abnormal PrP was rapidly taken up by finger-like projections called villi and passed in to the lymph. It was not, however, taken up by structures called Peyer's nodules, that are believed to be the places where animals amplify the infective agent. "The fact the PrP isn't taken up by the Peyer's nodules questions whether PrP is really infectious, or whether PrP is really just a secondary marker of the presence of the scrapie agent," says Jeffrey.

His belief in this need to reappraise the fundamental understanding of prion diseases is enhanced by one more observation published in this same paper. The team pre-digested a mixture containing disease specific PrP with standard stomach contents, and then injected the resulting mixture into the gut. No PrP transferred into the villi. When they used a highly sensitive version of Western Blot analysis to examine the contents of this pre-digested mixture, they found only the faintest suggestion that some of the PrP had survived. This was despite the fact that the original mixture had a contained a high level of PrP.

Dr. Martin Jeffrey about this said; "Think about it – a sheep grazing in a field is not naturally exposed to highly infected brain and could only pick up a tiny amount of PrP from other tissues. This will then be exposed to 48 hours or more digestion before it arrives in the gut, and our experiments show that after this, the chance of there being more than an unmeasurably small amount of PrP left to absorb is very small. As sheep can become infected, the theoretical probability of this being due to an invisible sub-fraction of digestion resistant PrP molecules is unlikely. The possibility of there being infectious molecules other than PrP must therefore be seriously considered. A lot of people are completely wedded to the prion hypothesis of diseases like vCJD, but the more you deal with whole animals as opposed to relying purely on in vitro studies, the more cautious you are about saying that prion proteins alone cause the disease,".

Although precious little is known about the transmission of vCJD, it is widely assumed that the disease is caused by eating beef from cattle with the BSE. The prion protein infection from transmissible BSE is then thought to travel to the brain via peripheral nerves, perhaps with assistance from the lymphoreticular system. Jeffrey´team research, has cast doubt on whether abnormal prion proteins are truly the infectious agents for vCJD disease infection in humans after all.

While their published research confirms that under laboratory circumstances prion-protein can be absorbed across the gut, it **also shows that this is unlikely to occur in real life.** In addition, the results show that the places in the gut that do take up these disease-associated proteins are different from the locations where infectivity is known to be amplified. This research questions whether prion protein is really infectious, and it **suggests that prion protein is merely a secondary marker of the presence of the scrapie agent.** If that is so, as their findings indicate, it might also be so for ostensibly infectious nature of prions in vCJD. That, however, leaves open the issue of what the disease's infectious agent might be. Roger Highfield in his report- article "Can this really kill you?"(May 30, 2006) wrote; The Nobel prize-winning hypothesis that infectious proteins can cause CJD and 'mad cow disease' is still being challenged…

2010; Surgery instruments and spontaneous prion generation also in Alzheimer's disease?

At first time team lead by COLLINGE (January 1997) showed a "Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy", and two years later started to be examined the third way of prion infection transmission, via surgical instruments. In article "Infectivity of scrapie prions bound to a stainless steel surface" (April 1999) researchers concluded; "The model system we have devised confirms the anecdotal reports that steel instruments can retain CJD infectivity even after formaldehyde treatment. It lends itself to a systematic study of the conditions required to effectively inactivate CJD, BSE, and scrapie agent adsorbed to stainless steel surfaces such as those of surgical instruments. In article "Transmission of scrapie by steel-surface-bound prions" (May 2001) researchers concluded; "Prions are readily and tightly bound to stainless steel surfaces and can transmit scrapie to recipient mice after short exposure times.

However, ten years later the same team headed by COLLINGE and WEISMANN **surprisingly found, according to the new research, wires coated with uninfected brain homogenate could also initiate prion disease in cell culture, which was transmissible to mice**. The catalyst in the study was the metallic surface of simple steel wires. Previous research showed that prions

bind readily to these types of surfaces and can initiate infection with remarkable efficiency. So "infectious" agents of the sort believed to cause mad cow disease in humans can appear "as if from nowhere" when healthy brain tissue comes into contact with steel. In article Spontaneous generation of mammalian prions **(published in PNAS, July 2010) researchers concluded**; "The apparent "spontaneous generation" of prions from normal brain tissue could result if the metal surface, possibly with bound cofactors, catalyzed de novo formation of prions from normal cellular prion protein. Alternatively, if prions were naturally present in the brain at levels not detectable by conventional methods, metal surfaces might concentrate them to the extent that they become quantifiable by the scrapie cell assay".

Scientists have shown for the first time that abnormal prions, bits of infectious protein devoid of DNA or RNA that can cause fatal neurodegenerative disease, can suddenly erupt from healthy brain tissue. Co-author of this study, Julie Edgeworth, stated: **"One theory for our observations is that the metal acts as a catalyst to promote the creation of prions from the normal prion protein present in brain tissue…"**

2. THE ORIGINS OF THE NEURODEGENERATIVE DISEASES MAY LIE IN CHRONIC MAGNESIUM DEFICIENCY COUPLED WITH A HIGH PROTEIN INTAKE

Presented as the poster No 132

Abstract

Epidemiological incidence of neurodegenerative diseases in a certain period, was only detected in cattle in the UK (BSE). This happened at a time, after significant increase of crude protein, in dairy rations (ARC, 1980) in the mid of 1980s, without equality of dietary Mg-supplementation. Feeding readily fermentable young grass (there especially most common ryegrass), leads to intraruminal ammonia concentrations (up to 30-70 mmol/l), and to decrease of magnesium absorption.

Neurodegenerative diseases, are caused by different mechanisms, but the common denominator of neuronal injury, is overstimulation of glutamate receptors. In excess, glutamate triggers a process called excitotoxicity, causing neuronal damage, particularly when NMDA receptors are activated. An

important consequence of NMDA receptor activation is the influx of Ca2+ into neurons, Mg2+ can protect against NMDA- induced neurodegeneration.

So the lower the Mg2+ level in the animal tissue cells, the more marked is "Ca-effect excitotoxicity". It should be noted that NMDA receptor channel in Purkinje cells, has a more extreme sensitivity to Mg2+, than that in other brain regions. Excitotoxicity can be found even with normal levels of glutamate, if NMDA receptor activity is increased, e.g., when neurons are injured-depolarized (more positively charged); this condition relieves the normal block of the ion channel by Mg2+, and thus abnormally increases NMDA receptor activity.

Well known is ammonia induced depolarization in cortical astrocytes, what results in removal of Mg2+, that normally blocks the NMDA receptor channel. Prolonged activation of NMDA receptors, was recently reported (Alzheimer´s research), to increase the neuronal production of amyloid β.

Introduction

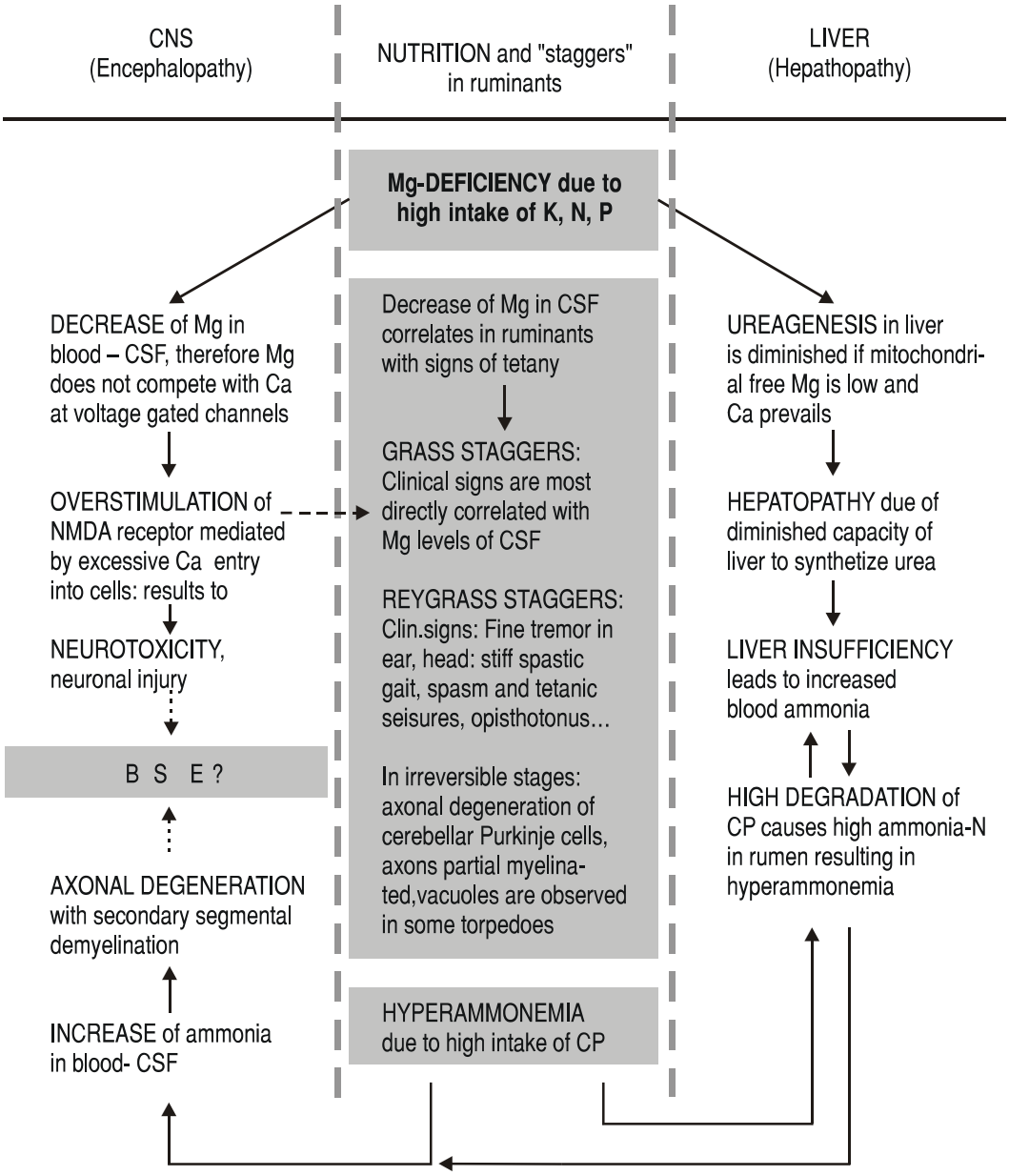
The neurodegenerative diseases, occurred to a greater extent (see UK; 1980s- 1990s), only in ruminants (BSE), because only in them, magnesium is not absorbed in the intestine, but in the rumen. In addition an excess of protein-nitrogen in the ruminants rumen decreases absorption of magnesium. The studies and findings reviewed in 1980- 1990s show that the surpluss of nitrogen and potassium in dairy cattle ration especially; can have the association with hyperammonemia complicated with the chronic subclinical hypomagnesiemia, and the neurodegeneration can be involved. On the other hand, despite extensive BSE research, there was much that was unanswered or mainly spekulative (e.g.see;The BSE Inquiry- October 2000). Therefore more of literature sources were reviewed about dairy cow nutrition and described as Czech alternative "BSE ammonia- magnesium theory" (March, 2001); in the Bulletin of Research Institute of Cattle Breeding, in Rapotín (see Fig 1).

Prion diseases are characterized by the replacement of the normal PrPc by a protease-resistant, sheet-containing isoform that is pathogenic (PrPSc). Pathology, in prion diseases, develops only in the brain, no other organ is affected. Early on neurons develop intracytoplasmic vacuoles. As the disease progresses, vacuolization becomes more pronounced and, microscopically, the cortical neuropil develops a spongy appearance, hence the term spongiform encephalopathy. Advanced cases show neuron loss, gliosis (astrocytosis), and brain atrophy. So as a result prions multiply, are not broken down by proteases and accumulate in brain tissue, where damage results by one of two mechanisms: (1) accumulation of the abnormal form of the **protein itself causes**

the damage ("vacuolization"); (2) the loss of function of normal protein results in cell death ("astrocytosis"). These both mechanisms can be involved by hypomagnesemia and hyperammonemia (FERRER, 2002).

Fig. 1

Nervous diseases and connections with nutrition in ruminants



CNS: Central nervous system • CSF: Cerebrospinal fluid • CP: Crude protein
NMDA: "N-Methyl-D-Aspartate" receptor.

The brain has two types of cells; neurons and glia. Neurons contain neurotransmitters, which are chemicals that trigger signals to pass messages. Until recently, neuroscientists believed neurons were the only brain cells transmitting message signals. Glial cells (astrocytes) were thought to serve only as support. Glia, once thought to simply provide structural support for their more important neuronal cousins, have been found, in the past decade, to have a wide variety of important biological functions. One of the most important of these is to foster a proper chemical environment for neuronal function by removing excess glutamate.

Why is this important? Because glutamate is a neurotransmitter, i.e., it can bind to receptors on the neuronal membrane and cause it to fire. Thus, glutamate is key to proper neurological functioning. Too much glutamate, however, is a problem, because it could cause neurons to work too hard, fatigue and die a premature death. This phenomenon is called glutamate toxicity.

Glutamic acid and glutamate receptors

Glutamic acid (Glutamate –"Glu") as the major excitatory neurotransmitter in the mammalian CNS; acts postsynaptically at several receptor types named for their prototypic pharmacological agonist. In excess, glutamate triggers a process called excitotoxicity, causing neuronal damage and eventual cell death, particularly when NMDA receptors are activated. This may be due to:

- High intracellular Ca2+ exceeding storage capacity and damaging mitochondria, leading to release of cytochrome and apoptosis,
- Glu/Ca^{2+}-mediated promotion of transcriptions factors for pro-apoptotic genes, or downregulation of transcription factors for anti-apoptotic genes.

There are 2 families of glutamate receptors located at the plasma membrane of neurons, ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors.The iGluR family is further divided into 3 classes of receptor, which are based on specific agonists and/or permeability to different cations; NMDA receptors (NR1, NR2A–D and NR3A–B) are predominantly Ca^{2+} ion permeable, whereas α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; GluR1–4) and kainate (KA; GluR5–7, KA1–2) receptors are predominantly permeable to Na^{+} and K^{+} ions (KEW and KEMP, 2005).

There are 8 metabotropic G-protein coupled glutamate receptors, which are classified on the basis of their structure and physiologic function into 3 distinct groups. Group 1 receptors (mGluRs 1 and 5) are coupled to phospholipase C through G_q and G_{11} proteins, whereas group 2 (mGluRs 2 and 3) and group 3 (mGluRs 4 and 6–8) receptors are coupled with adenylyl cyclase through G_i and G_o proteins (KEW and KEMP, 2005).

There is evidence that almost every step in the signalling pathways associated with mGluRs requires or is modulated by Ca^{2+}. Unlike iGluRs, which are

predominantly situated in the postsynaptic membrane to mediate fast excitatory transmission, mGluRs are located in various membrane compartments of both neuronal and glial cells in the brain (FERRAGUTI and SHIGEMOTO, 2006).

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).

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 that are most relevant to this review are the 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 NMDA receptor is extremely complex with a variety of means and conditions by which it mediates inter- and intra- cellular glutamate (Glu) transmission (NEWCOMER and KRYSTAL, 2001). It is known that activity stimulated by calcium influx through the NMDA receptor (NMDAr) is specific to the molecular environment in which it is located (HARDINGHAM and BADING, 2003). While there is still much more detail to learn about this, recent studies point to the location of the receptor on the neuron to explain its paradoxical role in both neuroprotective and excitotoxic cellular events. The work of HARDINGHAM et al. found that NMDA receptors located outside of the synapse lead to cell death whereas receptors located inside of the synapse lead to neurogeneration (HARDINGHAM et al. 2002).

Astrocytes regulate a release of glutamate

Astrocytes in the brain form an intimately associated network with neurons. They respond to neuronal activity and synaptically released glutamate by raising intracellular Ca concentration Ca2+, which could represent the start of back-signalling to neurons. 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).

Astrocytes establish rapid cell-to-cell communication through the release of chemical transmitters. The underlying mechanisms and functional significance of this release was, however, not well understood. BEZZI et al. (2004) identified an astrocytic vesicular compartment that is competent for glutamate exocytosis.

After activation of metabotropic glutamate receptors, astrocytic vesicles underwent rapid (milliseconds) Ca^{2+}- and the vesicular SNARE protein (cellubrevin) -dependent exocytic fusion that was accompanied by glutamate release. These data document the existence of a Ca^{2+}-dependent quantal glutamate release activity in glia that was previously considered to be specific to synapses (BEZZI et al., 2004).

Astrocytes express receptors for many neurotransmitters, and their activation leads to oscillations in internal Ca^{2+}. These oscillations induce the accumulation of arachidonic acid and the release of the chemical transmitters glutamate, D-serine, and ATP. The obtained evidence 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 Ca^{2+} 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, Ca^{2+}-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).

Ammonia is a neurotoxic substance which accumulates in brain in liver failure and it has been suggested that ammonia plays a key role in contributing to the astrocytic dysfunction characteristic of hepatic encephalopathy. In particular, 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 hepatic encephalopathy. CHAN et al. (2000) found that the reduced capacity of astrocytes to reuptake glutamate following ammonia exposure may result in compromised neuron-astrocyte trafficking of glutamate and could thus contribute to the pathogenesis of the cerebral dysfunction characteristic of hyperammonemic syndromes such as hepatic encephalopathy. An acute exposure to ammonia, resulting in cytosolic alkalinization (pH action), leads to Ca-dependent glutamate release from astrocytes. A deregulation of glutamate release from astrocytes by ammonia could contribute to glutamate dysfunction consistently observed in acute HE (ROSE et al., 2005).

In the 1980s; new NMDA receptor and a magnesium sensitivity was discovered

In the 1980s great strides have been made toward better undersanding the function of neurotransmitters, in particular because of the application of voltage – and patch- clamp techniques to cultured neurons that express the receptors and because of the development of specific receptor antagonists (COLLINGRIDGE and LESTER, 1989; MAYER and WESTBROOK, 1987; MONAGHAN et al.,1989; STONE and BURTON, 1988). This surge in information has not only resulted in a detailed understanding of the currents that underlie the fast excitatory amino acid- mediated transmission at many central synapses but has unveiled an exciting new receptor type, NMDA receptor, the activity of which is gated in a unique manner both by ligand binding and by voltage.

The normal voltage dependent block of channels by Mg^{2+} is highly important for the sort of functions NMDA receptors have to perform. The block is only relieved by an appropriately timed depolarisation of the neuron which may be brought about by activation of other types of glutamate receptor, including the fast acting AMPA subtype. Membrane depolarisation to remove the block of channels by Mg^{2+} is a prerequisite for NMDA activation and synaptic plasticity, whereas protracted or intense NMDA activation may cause neural injury such as follows cerebral ischaemia.

In the central nervous system (CNS) magnesium (Mg^{2+}) ion has two major functions: the stabilization of synaptic connectivity and widespread enhancement of neurochemical enzymatic functions. The Mg^{2+} has been shown to affect guanine nucleotide binding proteins (G proteins) in several ways: nanomolar concentrations of Mg^{2+} are required for GPT-ase activity (GILMAN, 1987: HIGASHIJIMA et al., 1987), micromolar concentrations of Mg^{2+} are required for receptor mediated activation of G proteins (GILMAN, 1987: GIERSCHIK et al., 1988), milimolar concentrations of Mg^{2+} increase the affinity of various types of receptors for agonists, an effect thought to result from increased receptor- G- protein coupling (HULUME et al., 1983), voltage-dependent- Ca^{2+} channel (AUGUSTINE et al., 1987), and NMDA receptor operated ionic channel (CRUNELLI and MAYER, 1984: NOWAK et al., 1984)

The NMDA receptor cation channel complex is unique in having several receptor and modulatory sites which recognise individual regulators found in the brain (REYNOLDS, 1990). These modulatory sites include an agonist binding site that binds L-glutamate and L-aspartate (MONAGHAN,COTMAN 1986), a glycine co-agonist site (REYNOLDS ET AL. 1987), a voltage-dependent Mg^{2+} site (NOWAK ET AL. 1984; JOHNSON, ASCHER 1990), a voltage independent inhibitory Zn^{2+} site (PETERS ET AL. 1987; REYNOLDS,

MILLER 1988) and a site which is affected by the endogenous polyamines spermine and spermidine (WILLIAMS ET AL. 1989).

The Mg^{2+} site is within the cation channel and is occupied at normal membrane potentials which inactivates the NMDA receptor. Depolarisation of neurons, which may be achieved by activation of other glutamate receptors such as the AMPA receptor, is sufficient to overcome the Mg^{2+} block and allow ion flow through the cation channel. Another cation channel site recognises phencyclidine (PCP) and other compounds, such as the dissociative anaesthetic ketamine and the anticonvulsant MK-801 (Dizocilpine). PCP-like compounds and MK-801 are non-competitive antagonists at the PCP site (FOSTER, WONG 1987; RANSOM, STEC 1988). The binding of [^{3}H]MK-801, which is determined by the open state of the cation channel and is increased by glutamate and glycine acting via their sites, is used to measure channel opening and access to PCP sites in vitro (REYNOLDS, MILLER;1988).

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 Mg2+ ion that blocks the channel from the outside. In neurons from spinal cord, 10 mM Mg2+ is required to block NMDA responses at potentials negative to 0 mV (MAYER and WESTBROOK, 1985). It may be that the NMDA channel in Purkinje cells has a more extreme sensitivity to Mg2+ than that in other brain regions.

Aspartate appeared to act at the NMDA receptor as well as at additional sites, since part of the aspartate response showed a Mg2+ sensitivity similar to that of the NMDA response (SEKIGUCHI et al., 1987). These results suggest that cerebellar Purkinje cell dendrites do posses NMDA channels, but their functional role was still unclear. The receptor pharmacology in cerebellum is apparently slightly different from other brain regions. First, APV does not entirely block NMDA responses (CREPEL et al., 1983; SEKIGUCHI et al., 1987). Second, aspartate seems preferentially to activate NMDA- like receptors, some of which mediate effects that are entirely blocked in 1 mM Mg2+ (SEKIGUCHI et al., 1987) and others at which NMDA may act as a competitive antagonist (CREPEL et al., 1983; KIMURA et al., 1985; SEKIGUCHI et al., 1987).

Neuronal free Ca concentrations correlates with the likelihood of irreversible ischaemic cell death (CHOI, 1985), and free intracellular Ca increases may result from Ca entry via the NMDA ion channel and voltage - gated Ca channels, and release from endoplasmic reticulum and other intracellular stores. Mg competes with Ca at voltage- gated Ca channels both intracellularly and on the cell surface membrane (ISERI and FRENCH, 1984). It may thereby impede Ca- dependent presynaptic release of glutamate and prevent neuronal Ca

overload via voltage- gated channels during ischaemia. Mg also enhances mitochondrial buffering of raised intracellular free Ca ions (FAVARON and BERNARDI, 1985), and prevents release of intracellular Ca stores from endoplasmic reticulum.

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 Mg^{2+} 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). An important endogenous allosteric inhibitor of NMDA receptor activation is pH. 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)

Magnesium - NMDA receptors; 20 years later and Alzheimer´s disease treatment

Magnesium helps maintain tissue sensitivity to insulin, control glucose metabolism, and is involved in insulin regulation. Magnesium deficiency leads to sugar and chocolate cravings that will disappear when normal levels are restored. Since significant levels of magnesium are found in the hippocampus, which is the emotional and memory center of the brain, deficiencies are believed to form the emotional environment that encourages cravings.

Putting into the human practical conditions, researchers have found that a new **highly absorbable form of magnesium called magnesium-L-threonate** concentrates more efficiently in the brain, rebuilds ruptured synapses, and restores the degraded neuronal connections observed in Alzheimer's disease and other forms of memory loss (SLUTSKY et al. 2010). In experimental models, **magnesium-L-threonate induced improvements of 18% for short-term memory and 100% for long-term memory.** Functionally, magnesium increased the number of functional presynaptic release sites, while it reduced their release probability. The resultant synaptic reconfiguration enabled selective enhancement of synaptic transmission for burst inputs. Coupled with concurrent upregulation of NR2B-containing NMDA receptors and its downstream signaling, synaptic plasticity induced by correlated inputs was enhanced.Putting into the practical conditions, researchers have found that a new highly absorbable form of magnesium called magnesium-L-threonate concentrates more efficiently in the brain, rebuilds ruptured synapses, and restores the degraded neuronal connections observed in Alzheimer's disease and other forms of memory loss. Functionally, magnesium increased the number of functional presynaptic release sites, while it reduced their release probability. The resultant synaptic reconfiguration enabled selective enhancement of synaptic transmission

for burst inputs. Coupled with concurrent upregulation of NR2B-containing NMDA receptors and its downstream signaling, synaptic plasticity induced by correlated inputs was enhanced (SLUTSKY et al. 2010).

Proteopathic Seeds and Neurodegenerative Diseases (Isabelle de SEGONZAC, February 2012)

In the mid 1980s, Stanley PRUSINER startled the scientific world by claiming that transmissible neurodegenerative diseases such as Creutzfeldt-Jakob in humans and Bovine Spongiform Encephalopathy (BSE; 'mad cow disease') were caused by self-replicating protein molecules, which he named prions. **Painstaking work to establish that prion proteins could replicate without the need for genetic material won him the Nobel Prize in 1997**. What at first seemed an unusual mechanism restricted to a rather rare group of diseases has now become central to the study of all neurodegenerative conditions: the pathogenic proteins that characterise these diseases **all seem to behave like prions.**

Prions are Janus-like proteins synthesised by neurons: in their normal, globular conformation they participate in cellular functions but in certain circumstances they adopt a pleated B-sheet configuration, which forms insoluble fibrous aggregates that disrupt cell function. This aggregated form is found in neurons in a group of neurodegenerative diseases known as the transmissible spongiform encephalopathies, which include Kuru, Creutzfeldt-Jakob disease (CJD) in humans, BSE in cattle and scrapie in sheep. All of these diseases can be transmitted by contact with brain material from affected individuals -- the cause of great concern in the late 1980s and early 1990s **when people developed a form of CJD after eating products from cows with BSE.**

By the 1980s, a long hunt had failed to find either a bacterial or viral agent causing these diseases. Stanley PRUSINER and his colleagues proposed instead that the **infectious agent was the B-sheet form of the prion protein**, which was able to replicate using itself as a template. As the first claim for replication without the need for nucleic acids, this was to say the least controversial. Now it is well accepted that 'rogue' molecules in the B-sheet conformation, **now known as 'prions', can act as a seed, converting normal prion proteins into B-sheet type molecules.** These adopt a fibrillar configuration and aggregate into an amyloid-like deposit that disrupts the neuron's function. Prions released from cells are taken up by neighbours and trigger the same cascade of transformation and aggregation. Genetics still plays a part, because various mutations in the prion protein gene promote this transformation, while some polymorphisms (substitution of one base in the gene sequence for another) make individuals more susceptible to developing a prion disease.

The parallels with Alzheimer's disease (AD) were soon noted: a cellular protein, in this case the amyloid-B peptide, adopts a B-sheet, fibrillar conformation that aggregates in the brain as amyloid plaques; again genetics plays a part, at least in early-onset, familial AD, which is associated with mutations in amyloid-B's parent protein, the amyloid precursor protein. More recently, it has **become clear that this prion-like pattern is common to all the neurodegenerative diseases,** including Parkinson's, Huntington's and motor neuron disease (Stanley PRUSINER, University of California San Francisco, San Francisco, USA): each is characterised by a disease-specific cellular protein that transforms into a B-sheet configuration that subsequently aggregates. Moreover, mutations associated with familial forms of the diseases have now been identified for all these signature proteins. As a consequence these conditions are now being designated as protein misfolding disorders (Claudio SOTO, University of Texas Houston Medical School, Houston, USA) and the proteins responsible could be considered as mammalian prions (PRUSINER).

If the misfolded proteins associated with the various neurodegenerative diseases do behave like prions, they should be **capable of triggering the transformation of the cellular protein in unaffected cells.** Transfer of a systemic (non-neural) amyloidosis between mice was first demonstrated over 40 years ago (Per WESTERMARK, Uppsala University, Uppsala, Sweden). Several speakers at the meeting have presented data supporting this hypothesis for various neurodegenerative diseases, **either by injecting a brain homogenate** from mice genetically engineered to develop the disease into the brains of susceptible but disease-free animals (PRUSINER; Mathias JUCKER, Hertie-Institute for Clinical Brain Research and German Center for Neurodegenerative Diseases, Tubingen, Germany; SOTO; Michel GOEDERT, MRC Laboratory of Molecular Biology, Cambridge, UK; Patrik BRUNDIN, Lund University, Lund, Sweden; Virginia LEE, University of Pennsylvania School of Medicine, Philadelphia, USA**); by injecting synthetic protein fibrils into brains** (LEE); **or by testing purified protein extracts on neuron cultures** (Anne BERTOLOTTI, MRC Laboratory of Molecular Biology, Cambridge, UK; Ron KOPITO, Stanford University, Stanford, USA). Another clear indication of transcellular induction comes from Parkinson's disease patients who have had stem-cell transplants: B-sheet proteins have been found in the neurons derived from the stem cells (BRUNDIN).

This triggering ability of the aberrant proteins, which has gained them the label of **proteopathic seeds, also seems to be responsible for the temporal spread of degeneration through the brain that is typical of the neurodegenerative diseases** (JUCKER; BRUNDIN; LEE). Perhaps more significant, the aberrant proteins have been found in the brain after intra-peritoneal injection or blood transfusion (SOTO); as with prions, transport along the vagal nerve seems to be the most likely route into the brain (PRUSINER; BRUNDIN). This opens up the

possibility of an environmental causation for the many patients with a neurodegenerative disease who do not have hereditary links (JUCKER; SOTO; WESTERMARK).

The **mechanisms underlying proteopathic seeding are still unclear**. The spread of the B-sheet transformation seems to depend on both the configuration of the seed itself and the genetic constitution of the animal -- again very like the prion diseases (JUCKERr; GOEDERT). The uptake of the seed proteins into neurons is being examined in culture (BERTOLITI; KOPITO) and model systems (BRUNDIN). **The key seems to be in the interaction between the seed protein and cell membranes** and, in some cases at least, helper proteins are required (BRUNDIN).

To understand how seeding works, it is essential to know the structure of the B-sheet proteins. Taking amyloid-B as an example, the conditions that determine what type of fibril and aggregates will form, and how this relates to the mutations in the amyloid precursor protein will be discussed (Robert TYCKO, National Institutes of Health, NIDDK, Bethesda, USA). Cooperativity between B-sheet molecules may also be important in aggregation (Roland RIEK, ETH Zurich, Zurich, Switzerland). Helpful insights can also come from systemic diseases in which amyloid accumulates, such as AA amyloidosis. Amyloid, a generic term for protein aggregates, is in this case produced by the inflammatory protein serum amyloid A (WESTERMARK). There is evidence that AA amyloid formation can be triggered by other types of amyloid molecule, leading to speculation that amyloid fibrils found in the environment and food could cross-seed amyloid formation in the body or brain.

As knowledge about proteopathic seeding accumulates, new prospects for therapeutic intervention open up (Peter LANSBURY, Brigham and Women's Hospital, Boston, USA). The initial conversion of functional globular protein into potentially pathogenic B-sheet form, the seeding cascade that coverts further globular protein to B-sheet, and the mechanisms by which neurons take up prion-like B-sheet molecules are all potential targets. **The discovery that amyloid-B seeds are partly soluble** and may be present in body fluids offers a possible alternative strategy for an early diagnostic (JUCKER).

At the same time, it is essential to remember that prion-like molecules have biological functions, which poses further challenges in the design of therapeutics. A form of amyloid seems to participate in the storage of hormones in secretory granules and in skin pigmentation (RIEK), while self-replicating prion-like proteins are a necessary part of the molecular mechanism for long-term memory storage in both the fly and the mouse (Eric KANDEL, Columbia University, New York, USA). Intervention to prevent the spread of a B-sheet protein like amyloid-B through the nervous system that interfere with important biological mechanisms, particularly those involved in memory storage, obviously need to be avoided. Despite these caveats, the unfolding of yet

another significant aspect of neurodegeneration offers exciting prospects for both the basic understanding of these devastating diseases and their treatment.

Prions and other Misfolded Amyloid Proteins

(Claudio SOTO, February 2012)

Misfolded protein aggregates are implicated in a variety of diseases known as **protein misfolding disorders (PMDs),** which include some highly prevalent and insidious illnesses such as Alzheimer's, Parkinson's disease, diabetes type 2 and more than 20 other human maladies. These diseases share as a hallmark event the misfolding of a protein to form ß-sheet rich aggregates that deposit in diverse organs inducing cell death and tissue damage. Among PMDs, prion disorders are unique in that the pathology can be transmitted by an infectious process involving a heretical agent known as prion. **Prions are infectious proteins capable to transmit biological information by propagation of protein misfolding and aggregation.** The hypothesis that prions are composed exclusively by a protein with the unprecedented ability to behave like a micro-organism was controversial during decades, but recent studies have settled all doubts. The process of misfolding and aggregation follows a seeding-nucleation mechanism in which small oligomers act as seeds to trigger an uncontrolled aggregation process. **The seeding mechanism appears to be the basis by which misfolded prion protein propagates prion disease in an infectious manner**. We have been able to create infectious prion protein in the test tube and produce a variety of novel diseases. So far, prion disease is the only member of the group of PMDs that has an infectious origin. However, the inherent **infectious nature of misfolded aggregates propagating by a seeding mechanism suggests that other diseases of the group may be transmissible in the same manner** as prion disorders. In this presentation, we will show evidences that the pathology of other PMDs, can be also experimentally transmitted in vivo. These findings may change our understanding of these diseases and provide novel strategies for treatment (SOTO, 2012).

To demonstrate a potentially infectious-like spreading of Alzheimer's disease in lab animals, SOTO and his fellow researchers(2010) injected mice with a small amount of brain tissue taken from a human with Alzheimer's, then compared the results to mice injected with normal brain tissue. None of the mice injected with the normal brain tissue showed signs of Alzheimer's, but **all of those injected with Alzheimer's brain extracts developed plaques and other brain alterations typical of the disease**, according to SOTO.

NMDA receptors as a common denominator of the neurodegenerative diseases; recent research

In the brain glutamate is used as a neurotransmitter in over 50% of the synapses in the neocortex of the frontal and temporal lobes. These are the areas of the brain where many neurodegenerative disorders are also found. The action of glutamate occurs at several receptors. The most common is the N-methyl-D-aspartate receptor. Glutamate, aspartate and glycine (dietary amino acids) can all stimulate this receptor. The NMDA receptor is a non-specific cation channel which can allow Ca2+, Na+, and K+ to pass into the nerve cell under normal conditions to facilitate neurochemical messages between cells. The net influx creates and excitatory post synaptic potential (EPSP) to occur between neurons. This is how nerve cells communicate in learning and memory and to initiate behavior. Mg2+ not only blocks the NMDA channel in a voltage-dependent manner but also potentiates NMDA-induced responses at positive membrane potentials. The reason Mg works, is that it blocks the NMDA receptor from firing constantly to cause neurons damage because of the low magnesium levels in those nerve cells. These findings are also seen in concussed depressed patients, those with diabetes, and those with AD or PD too. Na+, K+ and Ca2+ not only pass through the NMDA receptor channel in normal conditions but also modulate the activity of NMDA receptors. Its ion channel only opens when the following two conditions are met simultaneously: glutamate is bound to the receptor, and the postsynaptic cell is depolarized (which removes the Mg2+ blocking the channel). This specific property of the NMDA receptor explains many aspects of long term potentiation (LTP) and synaptic plasticity and it confers to humans the ability to learn and adapt (KRUSE, 2011)

Glutamate, NMDA receptors and Alzheimer´s disease

Alzheimer disease is characterized by 3 distinct major neuropathological abnormalities: intracellular neurofibrillary tangles, extracellular plaques and neuronal loss. Glutamate is the major excitatory neurotransmitter in the central nervous system and is known to be involved in a variety of functions, including synaptic transmission, neuronal growth and differentiation, synaptic plasticity and learning and memory. There is evidence that glutamatergic neurons located in the hippocampus and in the frontal, temporal and parietal cortex are severely affected, whereas similar neurons in the motor and sensory cortex are relatively spared (FRANCIS, 2003).

Amyloidogenic amyloid precursor protein (APP) processing

A large body of evidence has implicated amyloid beta (Aβ) peptides and other derivatives of the APP as central to the pathogenesis of AD. **Prolonged activation of NMDA receptors**, a situation analogous to Alzheimer disease pathology, increases amyloidogenic APP processing. When neurons are activated by high glutamate concentrations for longer time periods (i.e., more than 24 h) there is a "APP shift". This shift is mediated by activation of extrasynaptic NMDA receptors through Ca^{2+}/calmodulin-dependent protein kinase. The longer APP isoforms have been shown to be more amyloidogenic, and so this shift in expression increases amyloid β levels (BORDJI et al. 2010). Excessive glutamate release from the presynaptic terminals has also been suggested as a mechanism for increased amyloid β production via NMDA receptor–mediated Ca^{2+} influx (LESNÉ et al. 2005)

Glutamate-mediated synaptic plasticity and amyloid β peptide

Glutamate is essential in establishing new neural networks that form memories and assist learning through a **process called long-term potentiation**. This process is generated by high-frequency stimulation of the presynaptic plasma membrane, resulting in increased release of glutamate and activation of its receptors at the postsynaptic membrane. The AMPA and mGluRs are activated by the initial glutamate release, whereas NMDA receptors only become fully active after continuous synchronized glutamate stimulation following activation of the AMPA and mGluRs. **The NMDA receptor activation allows Ca^{2+} to enter the postsynaptic cell**, which subsequently triggers different kinase pathways and increases protein transcription. This process strengthens synapses and increases synaptic density (MORRIS, 2003). **Long-term depression occurs** when there is little stimulation at an established synapse or by asynchronous stimulation of iGluRs. Long-term depression has the opposite effect of long-term potentiation (i.e., reducing synaptic strength and preventing memory formation). **Long-term depression can be caused by a reduction in the levels of NMDA** and AMPA receptors at the postsynaptic membrane or by repeated weak stimulation of AMPA receptors that does not lead to the depolarization of the postsynaptic membrane and therefore does not fully activate NMDA receptors. This leads to a reduction in the number of neuronal spines on neurons and reduced synaptic activity. A decrease in long-term potentiation and an increase in long-term depression have both been observed in Alzheimer disease pathology (KULLMANN and LAMSA, 2007).

Many different paradigms have been used to show **that amyloid β disrupts long-term potentiation and reduces synaptic plasticity** (SHANKAR and MEHTA, 2008).

Glutamate and amyloid β–mediated toxicity

Chronic exposure to amyloid β peptides can induce toxicity in a variety of cell lines and in primary rat and human cultured neurons. The toxicity of the peptide is related to its ability to form insoluble aggregates (CLIPPINGDALE et al. 2001; PIKE et al 1993; SMITH et al. 2006)

However, recent evidence suggests that the **most detrimental forms of amyloid β peptides are the soluble oligomers** and that the insoluble amorphous or fibrillar deposits represent a less harmful inactivated form of the peptide (SELKOE and SCHENK, 2003).

The mechanisms associated with amyloid β toxicity are not clearly defined, but appear to involve alterations in intracellular calcium, production of free radicals, phosphorylation of tau protein and/or activation of caspase and noncaspase pathways that culminate in programmed cell death (CLIPPINGDALE et al. 2001; ROTH et al. 2001).

A number of studies have clearly indicated that **amyloid β toxicity is mediated**, at least in part**, by glutamate-mediated excitotoxicity**, which involves activation of the NMDA receptors, leading to elevated intra-cellular Ca^{2+} and consequent stimulation of a cascade of enzymes resulting in cell death (VOSLER et al. 2008; DONG et al. 2009).

Amyloid β–induced excitotoxic cell death was first reported in the early 1990s, with initial reports indicating that prolonged exposure of amyloid β peptides with glutamate induced greater cell death than exposure to amyloid β or glutamate alone in mouse cortical neuronal cultures (KOH et al. 1990).

The role of glutamate receptors in this process has subsequently been shown through the use of glutamate receptor agonists and antagonists. Recent data showing that **inactivation of the NMDA receptor by antagonists, such as MK-801, AP5 or memantine, can protect neurons from amyloid β toxicity** (TREMBLAY et al. 2000; SONG et al. 2002; FLODEN et al. 2005); that amyloid β induced neurodegeneration in the adult rat brain is mediated, in part, by activation of the NMDA receptor (HARKANY et al. 2000; MIGUEL-HIDALGO et al. 2002) and that transgenic mice exhibiting high levels of amyloid β peptide show increased vulnerability to excitotoxicity (GUO et al. 1999; MOECHARS et al. 1999).

Taken together, these studies suggest that **glutamate receptor activation may be essential for amyloid β–induced cell death.**

It is unclear how amyloid β peptides can regulate activation of glutamate receptors, leading to the death of neurons. There is evidence that amyloid β peptide can bind directly to glutamate receptors, increasing their activity and leading to influx of Ca^{2+} in organotypic slice cultures. Antagonists of NMDA and AMPA receptors can prevent influx of Ca^{2+} and cell death (ALBERDI et al. 2010).

Alternatively, it is possible that amyloid β–related peptides can increase extracellular glutamate levels by potentiating release (NODA et al. 1999; CHIN et al. 2007; KABOGO et al. 2010) and/or inhibiting the uptake of the neurotransmitter (HARRIS et al. 1996; FERNANDEZ-TOMÉ et al. 2004), which can subsequently trigger death of neurons by excitotoxicity. It is therefore likely that a combination of reduced glutamate clearance from the synaptic cleft, increased release of glutamate from neurons and glia, and the subsequent activation of the glutamate receptors contribute to toxicity mediated by amyloid β–related peptides.

MALINOW et al. 2013) found that glutamate binding to the NMDA receptor caused conformational changes in the receptor that ultimately resulted in a weakened synapse and impaired brain function.

They also found that beta amyloid; a peptide that comprises the neuron- killing plaques associated with Alzheimer's disease, causes the NMDA receptor to undergo conformational changes that also lead to the weakening of synapses.

NMDA receptors are well known to allow the passage of calcium ions into cells and thereby trigger biochemical signaling; new research, however, indicates that NMDA receptors can also operate independent of calcium ions. It turns upside down a view held for decades regarding how NMDA receptors function

Glutamate and amyloid β–mediated tau phosphorylation

Alzheimer's disease is characterized by the development of two different globs of proteins, **beta-amyloid plaques and neurofibrillary tangles- aggregates of a protein called tau**. Tau is normally a protein used in the cytoskeleton to build and maintain cellular structure. In the case of Alzheimer's Disease, tau proteins end up getting phosphorylated, have phosphorous attached to them, which causes them to be able to aggregate in groups, and if those get large enough, into neurofibrillary tangles.

Given the evidence that tau phosphorylation occurs before the loss of neurons and that inhibition of tau phosphorylation can prevent amyloid β–induced neurodegeneration (ALVAREZ et al. 2002; BRION et al. 2001; FERRER et al. 2005; ZHENG et al. 2002), it is likely that increased levels of phosphorylated tau can contribute to the death of neurons by triggering loss of microtubule binding, impaired axonal transport and neuritic dystrophy. A critical role of the phosphorylated tau protein in amyloid β–induced toxicity has been established

by the evidence that cells undergoing amyloid β toxicity exhibit increased levels of tau phosphorylation (ALVAREZ et al. 2002; BRION et al. 2001), that inhibition of tau phosphorylation by blocking tau kinases can prevent cell death (FERRER et al. 2005; STOOTHOFF et al. 2005), and that neurons cultured from tau protein knockout mice are resistant to amyloid β toxicity (RAPOPORT et al. 2002). It was found that glutamate receptor antagonists can protect neurons against amyloid β –mediated toxicity (FLODEN et al. 2005; SONG et al. 2008; TREMBLAY et al. 2000). Apart from influencing tau phosphorylation directly, there is evidence that Fyn kinase (fyn is a tyrosine-specific phospho-transferase that is a member of the Src family of tyrosine protein kinases) can regulate glutamatergic NMDA receptor activation following amyloid β treatment by triggering phosphorylation and subsequent interaction of the NR2B subunit of the receptor with PSD-95(postsynaptic density protein 95, a member of the membrane- associated guanylate kinase family, with PSD-93 it is recruited into the same NMDA receptor and potassium channel clusters). The NR2B/PSD-95/Fyn complex that is formed when NMDA receptors are activated perpetuates Ca^{2+} influx. It is therefore possible that NMDA receptor activation stabilizes the NR2B/PSD-95/Fyn complex, resulting in a persistent activation of the NMDA receptor channel and increasing tau phosphorylation (LEE et al. 2004).

Recently, a number of studies have indicated that caspase- and calpain-mediated proteolytic cleavage of tau protein, in addition to enhanced phosphorylation, may have a role in the amyloid β–induced degeneration of neurons. This proteolytic cleavage of tau may lead to neurodegeneration either directly, as reported in various cell lines and neurons, or indirectly by reducing the pool of full-length tau available for binding to microtubules (RAYNAUD and MARCILHAC, 2006). There is evidence that NMDA receptor–mediated activation of calpain may be involved in triggering the generation of tau fragments, which can lead to the degeneration of neurons, as both NMDA receptor antagonists and calpain inhibitors are found to protect neurons from cell death (AMADORO et al. 2006). Thus, NMDA receptor–regulated tau cleavage and hyperphosphorylation of the protein may represent 2 different mechanisms by which tau can cause cell death.

NOTE; Sometimes neurons can be surrounded by plaques and remain perfectly healthy. So in the opposing corner are scientists such as Rudy Castellani of the University of Maryland, who has denounced the beta amyloid hypothesis as "deeply flawed and certainly unproven." CASTELLANI (2010) has argued instead that tau, which in Alzheimer's disease forms abnormal tangles inside neurons, is more singularly important.

Taken together; the roles of amyloid β peptide in the function and control of the glutamate cycle and glutamatergic neurons;

- Inhibit long-term potentiation (SELKOE, 2008; LI et al. 2011; ZHAO et al. 2010; KLYUBIN et al. 2011)

- Increase long-term depression (KIM et al. 2001; HSIEH et al. 2006; LI et al. 2009)
- Decrease synaptic density and alter the morphology of dendritic spines (KNAFO et al. 2009; WEI et al. 2010)
- Regulate glutamate uptake from the synapse (FERNÁNDEZ-TOMÉ et al. 2004; SCOTT et al. 2011; MATOS et al. 2010)
- Stimulate release of glutamate (CHIN et al. 2007; KABAGO et al. 2010)
- Increase endocytosis of AMPA and NMDA receptors (HSIEH et al. 2006; OPAZO and CHOQUET, 2011)
- Disrupt the postsynaptic density and prevent NMDA and AMPA receptors reaching the cell surface (ROSELLI et al. 2009; GONG and LIPPA, 2010)
- Increase tau phosphorylation/cell death (FLODEN et al. 2005; SONG et al. 2008; TREMBLAY et al. 2000).

Glutamate antagonists in the treatment of Alzheimer disease

Owing to the critical role of glutamate in both amyloid β–and tau-mediated pathology, **glutamate receptor antagonists have been viewed as good therapeutic** targets for many years. Originally the noncompetitive NMDA receptor **antagonists MK-801 and phencyclidine** were tried as therapies for Alzheimer disease (DORAISWAMY, 2003). These compounds bind to sites within the NMDA receptor channel complex and prevent further Ca^{2+} entry by blocking the pore. Unfortunately, these drugs had a slow onset of action and bound irreversibly to the channel, preventing normal physiologic entry of Ca^{2+} into the cell. **This led to severe psychotomimetic side effects, such as hallucinations, ataxia and memory loss,** thus precluding their use as therapeutics for Alzheimer disease and also other conditions where NMDA receptors were therapeutic targets (FARLOW, 2004).

However, **another NMDA antagonist- memantine**, was found to be a noncompetitive, low to moderate–affinity NMDA receptor antagonist that can prevent pathological activation of the receptor **without affecting its physiologic functions** (REISBERG et al. 2003; SONKUSARE et al. 2005; LIPTON and CHEN, 2005; WENK et al. 2006). This antagonist exhibits a lower binding affinity than MK-801 and phencyclidine, but **has a higher affinity than the endogenous NMDA receptor antagonist, the magnesium ion, which normally blocks the voltage-dependent activation of the NMDA receptor.** Further studies indicate that memantine preferentially blocks NMDA receptor activity when the channel is excessively open. Since the NMDA receptor channels are open during normal synaptic activity for only milliseconds, memantine is unable to act or accumulate in the channel, thus selectively sparing

normal synaptic activity. However, during prolonged receptor activation, as occurs under prolonged depolarization or excitotoxic conditions, **memantine becomes an effective blocker of NMDA receptor activity** (PARSON and GILLING, 2007). These results convinced the European Union in 2002 and the U.S. Federal Drug Administration in in 2003 to **approve memantine for the treatment of moderate-to-severe Alzheimer disease.**

Altered neuronal calcium homeostasis and NMDA receptor antagonists action in AD was published by French scientists (BORDJI et al. 2010). They found that altered neuronal calcium homeostasis affects metabolism of amyloid precursor protein (APP), leading to increased production of Î¿-amyloid (AÎ¿), and contributing to the initiation of Alzheimer's disease (AD). A linkage between excessive glutamate N-methyl-d-aspartate (NMDA) receptor activation and neuronal AÎ¿ release was established, and recent reports suggest that synaptic and extrasynaptic NMDA receptor (NMDAR) activation may have distinct consequences in plasticity, gene regulation, and neuronal death. Altogether, these data suggest that a chronic activation of extrasynaptic NMDAR leads to neuronal AÎ¿ release, representing a causal risk factor for developing AD (BORDJI et al 2010). For one decade, several studies provided evidence that NMDAR activation could have distinct consequences on neuronal fate, depending on their location. Synaptic NMDAR activation is neuroprotective, whereas extrasynaptic NMDA receptors trigger neuronal death and/or neurodegenerative processes. Recent data suggest that chronic activation of extrasynaptic NMDA receptors leads to a sustained neuronal amyloid-Î¿ release and could be involved in the pathogenesis of Alzheimer's disease. Thus, as for other neurological diseases, therapeutic targeting of extrasynaptic NMDA receptors could be a promising strategy. Following this concept, memantine, unlike other NMDA receptor antagonists was shown, to preferentially target the extrasynaptic NMDA receptor signaling pathways, while relatively sparing normal synaptic activity. This molecular mechanism could therefore explain why memantine is, to date, the only clinically approved NMDA receptor antagonist for the treatment of dementia (BORDJI et al 2011).

The clinical effects of memantine have been widely studied, and a number of trials and meta-analyses have shown beneficial effects of the drug on the global status, cognition, behaviour and function of individuals with moderate to severe Alzheimer disease without any major adverse effects (van DYCK et al. 2007; WINBLAD et al. 2007).

Another a potential novel treatment for Alzheimer's disease is Latrepirdine (commercially as Dimebon). Latrepirdine treatment blocked NMDA receptor, high-voltage activated calcium channels (WU et al.2008)

More novel treatment for Alzheimer's disease is NitroMemantine

See; Aβ induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss (TALANTOVA et al. 2013)

The first experimental drug to boost brain synapses lost in Alzheimer's disease has been developed by researchers at Sanford-Burnham Medical Research Institute. The drug, called NitroMemantine, combines two FDA-approved medicines to stop the destructive cascade of changes in the brain that destroys the connections between neurons, leading to memory loss and cognitive decline.

The decade-long study, led by Stuart A. Lipton, M.D., Ph.D., professor and director of the Del E. Webb Center for Neuroscience, Aging, and Stem Cell Research, who is also a practicing clinical neurologist, shows that NitroMemantine can restore synapses, representing the connections between nerve cells (neurons) that have been lost during the progression of Alzheimer's in the brain. In their study, conducted in animal models as well as brain cells derived from human stem cells, Lipton and his team mapped the pathway that leads to synaptic damage in Alzheimer's. They found that amyloid beta peptides, which were once thought to injure synapses directly, actually induce the release of excessive amounts of the neurotransmitter glutamate from brain cells called astrocytes that are located adjacent to the nerve cells. Normal levels of glutamate promote memory and learning, but excessive levels are harmful. In patients suffering from Alzheimer's disease, excessive glutamate activates extrasynaptic receptors, designated eNMDA receptors, which get hyperactivated and in turn lead to synaptic loss.

Lipton's lab had previously discovered how a drug called memantine can be targeted to eNMDA receptors to slow the hyperactivity seen in Alzheimer's. This patented work contributed to the FDA approval of memantine in 2003 for the treatment of moderate to severe Alzheimer's disease. However, memantine's effectiveness has been limited. The reason, the researchers found, was that memantine (a positively charged molecule) is repelled by a similar charge inside diseased neurons; therefore, memantine gets repelled from its intended eNMDA receptor target on the neuronal surface. In their study, the researchers found that a fragment of the molecule nitroglycerin commonly used to treat episodes of chest pain or angina in people with coronary heart disease, could bind to another site that the Lipton group discovered on NMDA receptors. The new drug represents a novel synthesis connecting this fragment of nitroglycerin to memantine, thus representing two FDA-approved drugs connected together. Because memantine rather selectively binds to eNMDA receptors, it also functions to target nitroglycerin to the receptor. Therefore, by combining the two, Lipton's lab created a new, dual-function drug. By shutting down hyperactive eNMDA receptors on diseased neurons, NitroMemantine restores

synapses between those neurons. NitroMemantine brings the number of synapses all the way back to normal within a few months of treatment in mouse models of Alzheimer's disease.

Controversy over the Causes of Alzheimer's

Alzheimer's is caused by plaques and tangles in the brain, right? Wrong, says a group of researchers who believe that focusing on these abnormal proteins is keeping scientists from investigating other potential causes. In a 2009 (CASTELLANI et al) article in the Journal of Alzheimer's Disease, they wrote that the popular **hypothesis that beta amyloid (the protein found in plaques) causes dementia is flawed**. This flawed hypothesis is why potential Alzheimer's drugs continue to fail in clinical trials, they said, and has caused unnecessary suffering for patients and families.

Dr. CASTELLANI is Professor of Pathology and Director of Neuropathology at the University of Maryland. The two Editors-in-Chief of the "Journal of Alzheimer's Disease" Mark Smith and George Perry, are co-authors of the paper, and with Dr. CASTELLANI (2004), have long hypothesized that events earlier in the disease process, not beta amyloid, cause Alzheimer's. Their logic goes something like this:

1. Abnormal accumulations of beta amyloid (the protein in plaques) and tau (the protein in tangles) are not harmful, and are simply end-stage signs of earlier problems
2. Recent research indicates that beta amyloid may be protective – a normal immune response and an anti-oxidant
3. The accumulation of beta amyloid that can be seen at autopsy (and on new brains scans) is not well correlated with dementia
4. The focus on these abnormal proteins has crowded out funding needed to research other hypotheses.

Still the Main Hypothesis, At Least in Public

Dr. CASTELLANI and his co-authors are not alone in their thinking. Also many researchers think the amyloid hypothesis is wrong and that Alzheimer's research is headed in the wrong direction. So why is the amyloid hypothesis still the main theory in Alzheimer's research? "Based on my interaction with various neuroscientists and clinicians in the field, the dominant hypothesis -- the so-called amyloid cascade, now the synaptic abeta hypothesis -- is widely viewed as seriously flawed," says Dr. CASTELLANI. "Unfortunately, there is a lot of money and associated influence, as well as prestigious names and titles with a personal stake in the ultimate success of treatment efforts modeled after their preferred construct. Alzheimer's research involves selling ideas as much as (and more in my view) objective pursuit of knowledge. In this respect, the peer

review process is a bit of a farce, as it encourages fealty to existing ideas and hampers innovation, in spite of unending lip service paid to the latter."

And if there are doubts about the amyloid hypothesis, why do presentations and articles about Alzheimer's for a lay audience often present it as fact? "The popular press (major media outlets, many of them) will run articles, with or without schematic representations, along with quotes from esteemed researchers at the world's top institutions," says Dr. CASTELLANI. "They speak of (overly simplistic) removal of bad proteins, the exciting results from (hopelessly irrelevant) experimental models, economic burdens to society if something isn't done, anecdotal accounts of human intervention, etc, etc. All this, which taken together amounts to no more than snake oil in terms of a cure, permeates public thought and pretty soon everyone wants to be vaccinated [against beta amyloid]. Lost in the process is a hypothesis that is deeply flawed and certainly unproven."

A Lot at Stake; The amyloid hypothesis, right or wrong, is important because a large number of potential Alzheimer's treatments are based on it. If it's wrong, the hopes of patients and families will continue to be dashed, and millions of dollars will have been wasted on drug development.
Finally, if the amyloid hypothesis is wrong, then new brain scans that can measure amounts of amyloid aren't useful, and in fact may falsely diagnose someone with Alzheimer's. This would make efforts to detect "preclinical Alzheimer's disease" difficult.

If Not Amyloid, Then What? So if the amyloid hypothesis is wrong, where should Alzheimer's scientists focus? Dr. CASTELLANI argues for starting over with a much broader approach. "I think we have to throw the kitchen sink at the problem," he says. "Everything should be on the table, including a poly-therapy approach that encompasses multiple constructs and hypotheses."

Starting over sounds discouraging, but Dr. CASTELLANI seems confident that scientists will eventually find the cause of Alzheimer's, if only because of a lucky break. "The proof will be in the pudding," he says. "Sooner or later, there will be a breakthrough, and it will be by accident. At that point, the time and effort will be devoted to elucidating a mechanism that explains the accident, as the change in accepted science will have occurred by the force of empiricism."