

Fatal familial insomnia and the role of the thalamus in sleep regulation

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THE PRION DISEASES

Human prion diseases, also defined as transmissible spongiform encephalopathies, are rare fatal neurodegenerative diseases that include several clinical forms known as “kuru”, Creutzfeldt–Jakob disease (CJD), Gerstmann–Straussler–Sheinker syndrome (GSS), the so-called “new variant” CJD, and familial and sporadic fatal insomnia (FFI and SFI). Prion diseases may also occur in animals, such as in sheep (“scrapie”), deer (deer wasting disease), mink (mink encephalopathy), and bovines (the so-called “mad cow disease” or bovine spongiform encephalopathy) (Collinge, 2001). Prion diseases may be sporadic, genetic, and infectious, and are characterized by the accumulation of a pathogenic variant of the prion protein, which induces degenerative changes in the central (and sometimes the peripheral) nervous system according to still unclear pathogenic mechanisms.

The conversion of the naturally occurring prion protein (so-called cellular prion protein, or PrP^C) into a protease-resistant isoform (so-called PrP^{Res} or PrP^{Sc}, Sc meaning scrapie), which then accumulates in the brain, is considered a pathogenic step common to all of the prion diseases (Prusiner, 1987). The cellular prion protein is codified by the *PRNP* gene located on the short arm of chromosome 20. Its translated product is a 253-amino-acid structure that undergoes post-translational modifications at the amino- and carboxy-terminals, and glycosylation at two sites (Asn181 and Asn197). This glycoprotein weighs 33–35 kDa, is associated with the cellular membrane where it is attached to the outer cell surface with a glycosylphosphatidylinositol anchor, binds copper at an octapeptide repeat, and is expressed ubiquitously throughout

the central nervous system, particularly in the hippocampal formations. Expression patterns of PrP^C in the brain comprise expression in both neurons and glial cells (astrocytes and oligodendrocytes), a rostrocaudal gradient, and association with γ -aminobutyric acid (GABA)ergic neurons containing calcium binding proteins (Moleres and Velayos, 2005).

The principal neuropathological features of the prion diseases, varying in the different clinical forms, are neuronal loss, spongiform changes, and gliosis, associated with the deposition of variable amounts of PrP^{Sc} (Prusiner, 1987; DeArmond et al., 1998; Collins et al., 2004). Extracellular amyloid plaques are found in about 15% of brains with prion disease (Roberts and Clinton, 1992). According to the “prion only” pathogenic hypothesis (Prusiner, 1987), the key step in prion pathogenesis is represented by the conformational conversion of the normal PrP^C isoform into an abnormally misfolded protein PrP^{Sc}, which has an increased β -sheet (from 3% to 43%) and reduced α -helical (from 42% to 30%) content, reduced solubility, and is resistant to the action of proteases such as proteinase-K. This altered PrP^{Sc} isoform(s) is thought to be responsible for transmission of prion diseases to both humans and animals, but the presence of the naturally occurring PrP^C is necessary for establishment of the disease (Brandner et al., 1996). PrP^{Sc} has been indeed postulated to interact with the host's PrP^C, inducing the transformation of its α -helices into a β -sheet conformation and thus provoking the transformation of PrP^C into PrP^{Sc} (Pan et al., 1993; Ma and Lindquist, 2002).

The subsequent steps along the pathogenic cascade are, however, still incompletely understood, and the very function of PrP^C remains unclear at present. The accumulation and amyloid-like aggregation of

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PrPSc, possibly associated with the loss of the naturally occurring PrPC, has been shown to lead to mitochondrial dysfunction induced by oxidative stress (Choi et al., 1998) and to disturbances in intracellular calcium (Florio et al., 1998). Such metabolic alterations, misfolding of the abnormal PrP isoforms in the endoplasmic reticulum, or their impaired clearance by the ubiquitin–proteasome complex would then activate cell death pathways along the apoptotic cascade (apoptosis is known to occur in prion diseases; Dorandeu et al., 1998), and subsequent neurodegeneration.

These pathogenic problems notwithstanding, the “(prion) protein only” hypothesis has represented a remarkable reversal of the “nucleic acid only” paradigm for infectious diseases, as prions are thought to be devoid of nucleic acid and infectivity in prion diseases resides within the prion protein itself. The argument that this “prion protein only” mechanism could not account for the variable features of the clinical diseases and infectivity experiments collapsed when it was shown that infectious prions come in different strains according to the conformational species of a particular prion encoded by the sequence of the PrP gene of the infected animal. Thus, the “prion protein only” hypothesis is also able to account for the variety of the pathological and clinical features found in the different prion diseases, which had previously been thought impossible to explain without the help of information from nucleic acids.

FAMILIAL FATAL INSOMNIA

Clinical features

FFI was first identified in 1986 in an Italian family as an autosomally dominant transmitted disease characterized by peculiar alterations in sleep and autonomic functions, and in which neuropathological examination disclosed prominent thalamic and inferior olivary changes (Lugaresi et al., 1986). FFI is a disease with onset usually in mid life: mean age at onset is 51 years, but the onset may range from 36 to 62 years. FFI affects both sexes equally, leading to death within a mean disease course of 18 months (range 8–72 months) (Lugaresi et al., 1986; Montagna et al., 1998). Two different disease courses are described, and attributed to the effects of the 129 *PRNP* codon polymorphism, which seems to modulate not only the disease duration but also the clinical features (see below); patients may have a short (less than 11 months; mean 9.1 months) or a prolonged (more than 11 months, mean 30.8 months) disease duration.

FFI was recognized as a genetically transmitted prion disease in 1992 (Medori et al., 1992a, b) and represents the third most frequent genetic prion

disease. It has been reported worldwide, and its clinical and pathological features remain comparable in populations with different genetic backgrounds (Nagayama et al., 1996; Almer et al., 1999; Harder et al., 1999; Tabernero et al., 2000). FFI is fortunately a rare disease, having been reported in about 27 kindreds up to now.

The disease usually runs clinically defined phases. The initial phase usually starts with subtle changes in sleep, vegetative functions, and personality, so subtle as to be often neglected by the patient and physician or to be trivialized into manifestations of stress or overwork. Patients complain that sleep is no longer satisfying or refreshing, that they have increasing difficulties in falling and remaining asleep, and can no longer nap in the afternoon. They feel fatigued and may look somnolent in the daytime, yet are unable to fall asleep and maintain sleep. From the outset, autonomic symptoms are often associated with these sleep abnormalities, characterized by slightly increased body temperature, especially in the evening, tachycardia and irregular rapid breathing, and impotence in males. Blood pressure also increases, and there is perspiration, salivation, and tearing. Patients are reported by relatives as apathetic or uninvolved, but intellectual functioning generally remains unaffected.

The sleep and autonomic alterations increase progressively, until peculiar behavioral abnormalities appear, consisting of dream enactment suddenly arising out of wakefulness, especially when the patients are left unstimulated. During such “oneiric stupors”, patients lapse into a state of unresponsiveness and perform complex gestures that, upon awakening and according to the patient’s report, appear to be well correlated to the content of a dream (Figure 59.1). These “oneiric stupors” increase progressively in frequency and duration, while recall of the dream content becomes progressively more difficult; at this point hypertension and other autonomic changes are usually well developed.

The middle phases see the onset of motor impairment, in the form of spontaneous and stimulus-evoked myoclonus, pyramidal system involvement, dysmetria and ataxia, and dysarthria with dysphagia.

In the final phases of the disease, patients become progressively bedridden and finally end up in a state of akinetic mutism, with stupor and coma preceding death. Death, however, may supervene suddenly in every phase of the disease, even when motor abnormalities are absent, and especially in patients whose disease has a short course.

These symptoms and signs are those most frequently observed and typical of FFI, and resemble those historically reported in the so-called thalamic

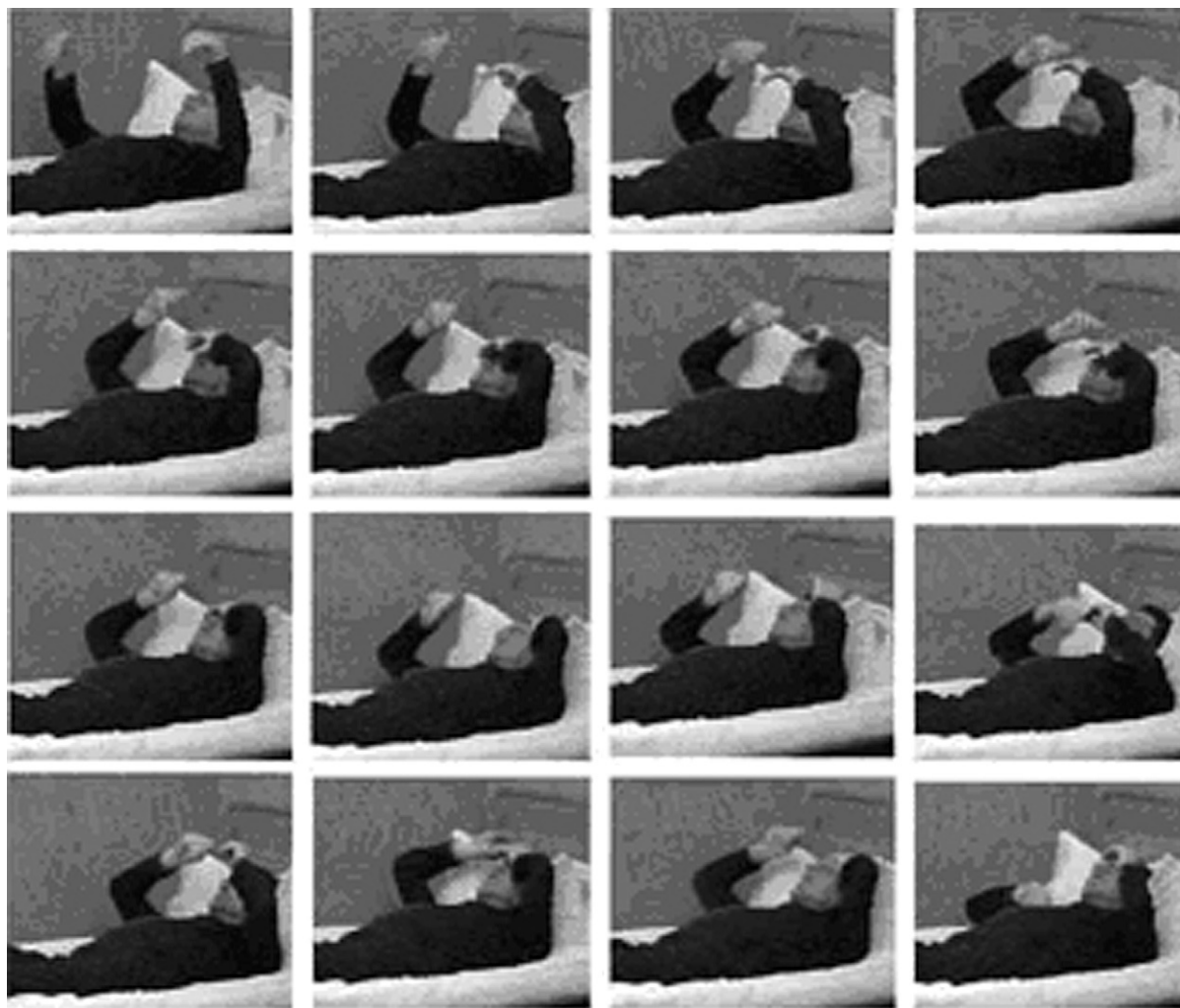


Fig. 59.1. Sequential frames recorded in a patient with familial fatal insomnia, showing complex semi-purposeful movements during an oniric stupor episode.

dementia (Petersen et al., 1992). They are by no means universal, however, and FFI kindreds display a degree of clinical variation, often related to the length of the disease. Patients with a short disease course manifest more marked sleep and autonomic abnormalities, whereas those with slower disease have more evident motor abnormalities (sometimes present even at the onset of the disease) and may suffer convulsive seizures. In some kindreds, moreover, overlap of clinical features has been reported, with siblings manifesting a full spectrum of phenotypes from typical CJD to FFI, to cerebellar ataxia without insomnia (McLean et al., 1997; Zerr et al., 1998b; Rossi et al., 1998; Taniwaki et al., 2000). Phenotypic heterogeneity has even been reported for half-brothers with the FFI mutation (Johnson et al., 1998). Although a genetic etiology (the 129 *PRNP* codon polymorphism) for the different duration of the disease has been postulated, there must

surely be other still unknown reasons, both genetic and nongenetic, for the observed clinical variability.

Clinical laboratory findings

Electroencephalographic (EEG) and polysomnographic recordings performed at various times during the clinical course of the disease in FFI show several remarkable changes in sleep macro- and micro-structure and in the organization of the circadian rhythms. A characteristic feature is the early disappearance of sleep spindles and K-complexes, which is readily evident upon spectral EEG analysis with loss of power in the sigma band (Sforza et al., 1995). The loss of sleep spindles is particularly evident in patients with a short disease course, but is eventually brought about in the course of FFI. Loss of spindles is permanent and spindles cannot be evoked even by the administration of

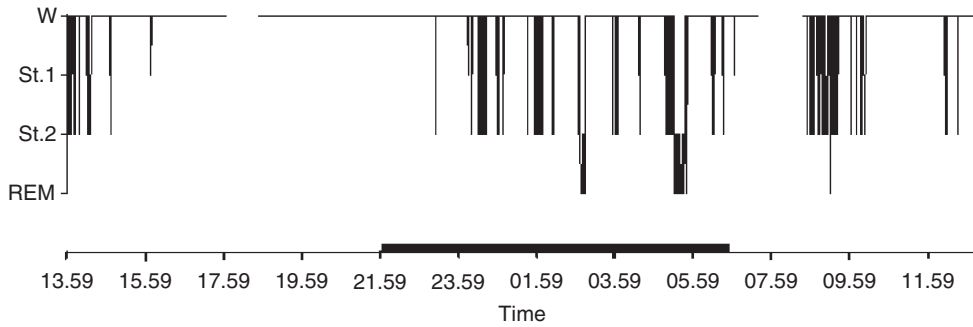


Fig. 59.2. Sleep histogram recorded in a patient with familial fatal insomnia at an early stage of the disease, showing the lack of deep phases 3–4 NREM sleep and a reduction in REM sleep, associated with frequent arousals and loss of the normal cycling of sleep. At this stage of the disease, the sleep spindles were still recognizable and there were no dissociated polysomnographic features. Black bar denotes dark period.

barbiturates or benzodiazepines, drugs that usually provoke spindling on the EEG (Tinuper et al., 1989). Likewise, barbiturates and benzodiazepines cannot induce slow-wave sleep (SWS). In fact, the next striking abnormality consists of the progressive loss of SWS, which characterizes deep nonrapid eye movement (NREM) sleep stages (Sforza et al., 1995) (Figure 59.2). Thus, EEG is characterized in the early–middle stages of FFI, by a pattern of alternation between an alpha activity of wakefulness mixed with theta trains associated with a waking behavior and normal response to external stimuli, and a desynchronized EEG activity with rapid eye movements (REMs), an often incomplete loss of antigravitary muscle tone, irregular muscle jerks, and autonomic fluctuations with tachycardia, tachypnea, and irregular breathing.

Behavior during the latter periods is peculiar, characterized by unresponsiveness to presented external stimuli and by the subsequent report of a dreaming mentation, which the patient is at first able to report upon awakening and which can be often observed to correspond closely to the quasi-purposeful gestures and jerks enacted during the state. As patients can be woken up easily, at least in the initial stages, these states were termed “oneiric stupors” (Tinuper et al., 1989). Thus, florid stages of the disease are characterized mainly by two states of vigilance, alternating throughout the 24 hours. The cyclic structure of sleep is consequently lost, and short periods of REM sleep and SWS can often be seen to arise directly from a state of wakefulness, associated with absent or minimal antigravitary muscle atonia or with a decrease of body core temperature in the case of REM sleep, or a buildup of EEG delta activity in the case of SWS periods (Figure 59.3). Eventually, in some patients, both SWS and REM sleep may completely disappear. This pattern is especially evident in patients with a short disease course, whereas those with longer disease may

conserve some deep sleep and cyclic alternation of the sleep stages for some time.

Later stages of the disease develop a monomorphic, low, unreactive EEG activity, with tracings becoming flat in the periods before death. During such activity, the patient is akinetic and unresponsive, and animated by the presence of isolated segmental or diffuse, spontaneous and evoked myoclonic jerks. Remarkably, the periodic spike discharges that characterize the EEG in prion diseases are nearly always absent in patients with a short disease course, and may be present in the more prolonged disease courses just a few days or weeks before death. Autonomic tests disclose alterations concomitant with the sleep changes, consisting of sympathetic activation (increased heart rate and blood pressure, increased breathing rate, increased body core temperature) both at rest and upon stimulations such as the Valsalva maneuver, isometric handgrip, or the tilt test. Parasympathetic functions remain unaffected (Cortelli et al., 1991). Autonomic features can be seen to have a state-dependent modulation, sympathetic activity decreasing whenever short periods of SWS appear on the EEG. They are attended by increased plasma levels of catecholamines, such as norepinephrine and epinephrine, and are associated with endocrine changes with hypercortisolemia in the presence of normal or even low levels of adrenocorticotrophic hormone (Portaluppi et al., 1994a; Montagna et al., 1995). Abnormal plasma levels of catecholamines and sympathetic activation are associated with loss of their circadian rhythmicity (Avoni et al., 1991) with, in particular, disruption of somatotropin secretion (Portaluppi et al., 1995). The nocturnal rise in melatonin is lost with disease progression (Portaluppi et al., 1994b). Actigraphic recordings in a patient with FFI with a short disease course also demonstrated the loss of any circadian rhythmicity in motor activity, showing persistently 80% increased motor activity. In this patient,

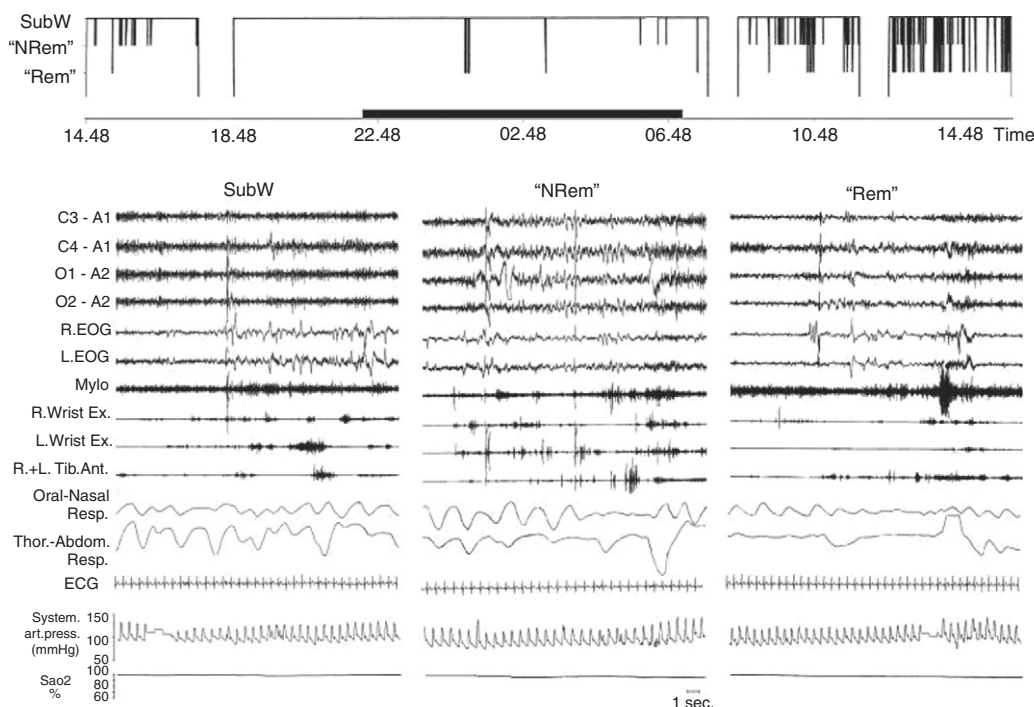


Fig. 59.3. (A) Sleep histogram and (B) excerpts of related polysomnographic (PSG) recordings in a patient with familial fatal insomnia at an advanced stage, showing lack of slow-wave sleep and brief, often clustered, episodes of REM sleep (on the histogram). Black bar denotes dark period. PSG excerpts demonstrate the dissociated features of the “subwakefulness” state (SubW) (mixed alpha–theta EEG activity, irregular jerky and more tonic muscle activity), the “NRem” light sleep (mixed alpha–delta EEG activity, irregular muscular jerks, irregular antigravitary muscle tone, eye movements, and irregular breathing and heart rates; absent sleep figures such as sleep spindles and K-complexes), and “Rem” sleep (lack of complete antigravitary atonia, limb muscle irregular jerks interspersed with more tonic activity). ECG, electrocardiogram; EOG, electro-oculogram; resp., respirogram; Sao₂, oxygen saturation; TA, thoracoabdominal; R, right; L, left.

recordings in an isolated respiratory chamber demonstrated a 60% increase in metabolic consumption, indicating a very high catabolic rate (Plazzi et al., 1997). Dissociation between conserved rest–activity and absent hormonal circadian rhythms (melatonin and cortisol) is also possible in FFI (Dauvilliers et al., 2004). Routine examination of the cerebrospinal fluid (CSF) is unremarkable in FFI, and levels of hypocretin-1 are essentially normal (Martinez-Rodriguez et al., 2003; Dauvilliers et al., 2004). Increased levels of the 14-3-3 protein in the CSF, a hallmark of sporadic CJD and other prion diseases, is not particularly helpful in the diagnosis, being found in only 50% of FFI cases (Zerr et al., 1998a).

Neuropsychological studies in affected individuals show alterations in vigilance, attention, working memory, and temporal orientation (Gallassi et al., 1992, 1996). Frontal executive functions are those prevalently affected. Patients perform quite well on global intelligence tests until and unless vigilance levels are markedly disrupted. Therefore, because of the important vigilance abnormalities, which should, in principle, be absent in true dementias, FFI represents a progressive

confusional–oneiric state rather than a true dementing illness (Gallassi et al., 1996). Nonaffected carriers of the FFI *PRNP* mutation, followed sequentially with a battery of neuropsychological tests in order to disclose any initial intellectual change, did not demonstrate any significant abnormality in the tests or any mental impairment prior to the clinical onset of disease as defined by the symptoms and signs of insomnia (Cortelli et al., 2006).

Neuroimaging findings

Brain metabolism in FFI was assessed by means of [¹⁸F] fluorodeoxyglucose positron emission tomography (18F-FDG-PET) in affected patients at different times in their clinical course (Perani et al., 1993; Cortelli et al., 1997). In stages of the disease when patients display severe polysomnographic alterations with oneiric stupors and insomnia, [18F]-FDG-PET demonstrates severe hypometabolism of the thalamus bilaterally, often associated with some hypometabolism of the gyrus cinguli. This thalamic hypometabolism may also be accompanied in later stages by hypometabolism of

the basal ganglia, especially the caudate, cerebellum, and cortex, especially frontotemporal areas. When motor disability becomes prominent, hypometabolism is always more widespread and affects, besides the thalami and the basal ganglia, all cortical regions except the occipital lobe, which is peculiarly preserved in FFI. Cortical hypometabolism outside the frontal limbic areas is more evident in patients with a prolonged disease course.

From a metabolic point of view, therefore, FFI is a predominantly thalamolimbic hypometabolic state; this characteristic metabolic “signature” of FFI has been found even in patients displaying atypical clinical symptoms (Bar et al., 2002), and is thus a helpful sign in the differential diagnosis. Thalamic hypometabolism may also be a marker in preclinical carriers of the FFI mutation, as a study of presymptomatic carriers of the 178 *PRNP* codon mutation followed longitudinally with 18F-FDG-PET confirmed that the first alteration in brain metabolism to be found and detectable 13 months prior to disease onset (as defined by symptoms and signs of insomnia) was thalamic hypometabolism (Cortelli et al., 2006); remarkably, in these patients limbic areas and the basal ganglia became hypometabolic too, but only after the clinical onset of the disease, whereas the neocortical regions never became significantly hypometabolic, even 10 months after disease onset (Cortelli et al., 2006) (Figure 59.4). A distinct temporal pattern of spreading brain metabolic changes is thus present in FFI, whereby the thalamus becomes hypometabolic even before symptom onset, and is in turn followed by the limbic areas and basal ganglia, whereas neocortical regions become hypometabolic only late in the disease course, if at all.

Single-photon emission computed tomography with 2- β -carbomethoxy-3- β -(4-iodophenyl)-tropane in two

patients with FFI disclosed abnormal availability of the serotonin transporter in the thalamus–hypothalamic region (reduced by 57–73%), which was thought to be secondary to the peculiar sleep abnormalities in FFI (Kloppel et al., 2002).

Neuropathological aspects

Neuropathological examination demonstrates severe changes in the thalamus in FFI. These consist of marked (up to 80%) loss of neurons, especially in the anterior ventral and the mediodorsal nuclei, associated with reactive astrogliosis (Lugaresi et al., 1986; Manetto et al., 1992). The changes are much less evident, and sometimes absent, in the other thalamic nuclei, the pulvinar excepted. Spongiform changes, the hallmark of prion diseases, are not usually found in the thalamus, but are encountered in the neuropil and the cerebral cortex, especially in layers II–IV, particularly in patients with a prolonged course. Indeed, gliosis and some spongiosis of the entorhinal cortex are the only changes found in the cerebral cortex of patients dying with a short disease course, whereas gliosis and spongiosis become more prominent and affect all lobes in patients who die after a prolonged course.

Other less consistent changes are gliosis and loss of neurons (Purkinje and granule cells) in the cerebellum, and occasionally in the basal ganglia and the nucleus basalis of Meynert. Only gliosis, but no neuronal loss, is found in the hypothalamus and the periaqueductal gray.

The other consistent change characteristic of the disease is marked loss of neurons in the inferior olivary nuclei, to a degree comparable to that in the thalamus, which thus makes FFI a preferential thalamic–olivary atrophy on neuropathological grounds. Although

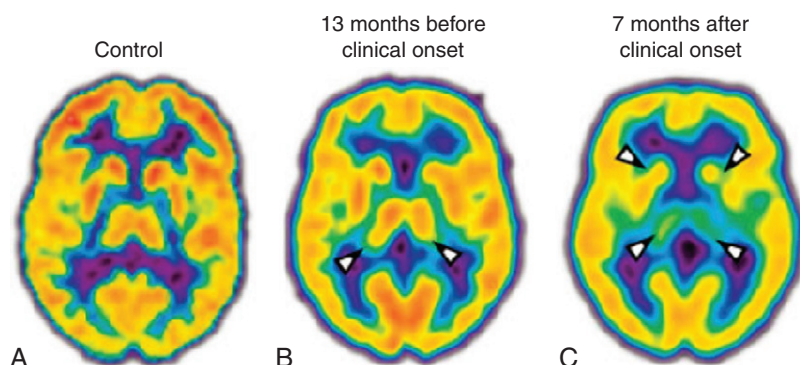


Fig. 59.4. [^{18}F]fluorodeoxyglucose positron emission tomography in a control subject (A) and a patient with familial fatal insomnia (B & C), showing thalamic hypometabolism (arrows in B) 13 months prior to disease onset as defined by the symptoms of insomnia. In addition to the thalamus, limbic areas and basal ganglia show hypometabolism but only after the clinical onset of the disease (arrows in C), whereas neocortical areas remain normometabolic even after disease onset. (From Cortelli et al., 2006, with kind permission. ©Oxford University Press.)

atrophy of the anterior ventral and mediodorsal thalamic nuclei together with the inferior olives is found in all cases of FFI, the changes in the basal ganglia and especially those in the cerebral cortex depend upon the length of the disease, being more evident the more prolonged the duration.

A noteworthy consideration is that FFI displays neuropathological changes distinct from those seen in the more common forms of prion disease, especially sporadic CJD. Specific features of FFI are the selective atrophy of the mediodorsal nucleus, and the loss of parvalbumin-positive GABAergic interneurons in the thalamus (Macchi et al., 1997; Guentchev et al., 1999; Masullo and Macchi, 2001). Serotonergic systems also seem particularly vulnerable in FFI, as shown by immunohistochemical studies of median raphe nuclei in FFI brains (Wanschitz et al., 2000) and by studies of serotonin metabolites in the CSF (Cortelli et al., 2001).

As befits a prion disease, the brain in FFI accumulates the typical protease-resistant PrPSc deposits (Parchi et al., 1995). Peculiar to FFI brains is that these deposits of PrPSc are scant, the amount being only 10–20% of that observed in sporadic CJD. Remarkably, the amount of PrPSc in the thalamus is particularly small, while deposits are found especially in the cortex and in patients with a more prolonged disease course. Indeed, with disease progression, deposits in the cerebral cortex (and basal ganglia) increase, whereas those in the thalamus and the brainstem remain uncorrelated to disease duration, showing a kind of ceiling effect. Deposits of PrPSc are more widespread than the histopathological changes of neuronal loss and astrogliosis, and there is no relationship between neuronal loss and PrPSc deposits. This suggests that the thalamic, especially the mediodorsal, nuclei, where the dissociation (severe neuronal loss in the face of scarce PrPSc) is particularly evident, have a specific vulnerability in FFI (Parchi et al., 1995).

Molecular neurobiology

FFI is transmitted as an autosomal dominant disease due to a mutation in the *PRNP* gene, whereby a GAC→AAC transition results in a D178N substitution at codon 178 of the gene (Medori et al., 1992a, b). The same missense mutation is, however, found in some pedigrees of CJD that have clinical and neuropathological features different from those in FFI, namely a lack of sleep changes and a preferential involvement of the cerebral cortex rather than the thalamus (Goldfarb et al., 1991). However, a detailed genetic analysis of these FFI and CJD pedigrees, both harboring the 178 codon mutation (178CJD), showed that all FFI kindreds are characterized by the presence

of a 129 codon polymorphism coding for the amino acid methionine on the mutated allele, whereas the 178CJD kindreds all express valine (Goldfarb et al., 1992). Thus two distinct phenotypes, FFI and 178CJD, are linked to the same mutation but to a different intragenic polymorphism: the 178 *PRNP* codon mutation determines disease, but the histopathological and clinical features are dictated by the 129 codon polymorphism present on the mutated allele (Petersen et al., 1994). The modifying effects of the 129 codon polymorphism were further confirmed in a FFI kindred harboring another intragenic polymorphism, a 24-base pair *PRNP* deletion (Bosque et al., 1992), and in a general review of the phenotypic spectrum of prion diseases (Kovács et al., 2002).

The 129 *PRNP* codon polymorphism is known also to exert modifying influences on the clinical characteristics of other prion diseases, in particular on the susceptibility to iatrogenic and “new variant” CJD, and on age of disease onset (Puoti et al., 2000; Collinge, 2001). In Caucasians, the 129 codon of the *PRNP* gene specifies either methionine or valine, with 34–49% of subjects being met/met homozygotes, 42–56% met/val heterozygotes, and the remainder val/val. How the 129 codon exerts these modifying effects is still unknown; the 129 valine genotype has been linked to increased PrPRes production, and 129 heterozygosity to increased amyloid plaque aggregation (Hauw et al., 2000). Monari et al. (1994) demonstrated that the genetic differences between FFI and 178CJD were also reflected in the type of PrPSc being accumulated in the brain: whereas FFI brains accumulated a 19-kDa PrPSc, 178CJD brain deposits were of a 21-kDa PrPSc. These different isoforms of PrPSc were also characterized by different glycosylation patterns; PrP isoforms can be un-, mono- and di-glycosylated, and the unglycosylated form is particularly scant in FFI. The different molecular weight of the two PrPSc, the FFI and 178CJD PrPSc, is explained by a different site of cleavage by proteinase-K, probably due to a different conformation of the two protein isoforms. The 21-kDa PrPSc was defined as type 1 and the 19-kDa isoform as type 2 PrPSc; this was the first demonstration of different types of PrPSc with distinct biochemical and pathogenic properties (the prion “strains”).

That the clinicopathological features of FFI and other prion diseases were indeed pathogenically related to the particular type of PrPSc isoform (“prion strain”) accumulated was demonstrated conclusively by experimental transmission studies performed in transgenic animals. Prion diseases are indeed infective and can be transmitted to experimental animals, as clearly shown by Collinge et al. (1995) and Tateishi et al. (1995). Remarkably, when extracts from FFI brains

were inoculated into transgenic mice (MHu2M) expressing a chimeric human–mouse PrP gene, mice developed a prion disease characterized by prominent thalamic pathology and the accumulation of a prion strain (PrPSc) similar in weight to that found in the donor patient brain, a 19-kDa type 2 PrPSc (Telling et al., 1996). In contrast, when brain extracts inoculated into the same transgenic mice came from patients with sporadic CJD or harboring an E200K codon *PRNP* mutation, the mice developed a prevalent cortical pathology and accumulated a type 1 PrPSc. This demonstrated that the conformation of the PrPSc acts as a template for the formation of nascent PrPSc molecules, and confirmed that prion strain diversity (and consequently their ability to induce different clinicopathological features) is encrypted in the particular conformation of any specific PrP isoform, with no participation from the donor DNA.

These results were instrumental in confirming the “prion protein only” theory of prion pathogenicity. Transmission experiments with extracts from SFI (see below) brains gave further support to this theory (Mastrianni et al., 1999). It is currently thought that PrPC, for some unknown reason and when mutated, undergoes a conformational change that makes it into a proteinase-K-resistant isoform that acts as a template to convert nascent PrP molecules into PrPSc ones (Prusiner, 1987). How this leads to loss of neurons and reactive astrogliosis in specific brain structures cannot, however, yet be explained satisfactorily. The D178N *PRNP* mutation results in abnormal intracellular transport of the protein (Petersen et al., 1996) and there is evidence for neuronal apoptosis in FFI brains (Dorandeu et al., 1998), but the intermediate pathogenic steps remain unclear. In particular, the specific vulnerability of the thalamolimbic structures remains unexplained.

The mechanisms behind the effects of the 129 codon polymorphism are also puzzling. It was hypothesized that, because the 178 and 129 residues are close in the tertiary structure of the protein, different interactions between valine and methionine residues resulted in a different protein conformation (or isoform) (Parchi et al., 1998). More recently, using molecular dynamics, Shamsir and Dalby (2005) examined the effects of both the codon 178 mutation and 129 codon polymorphism on protein dynamics and conformations. They found that methionine variants had higher stability than valine variants, and that elongation of β -sheets and new β -sheet formation occurred more readily in valine variants. The conversion propensity for the D178N variant has also been shown to depend strongly upon the M/V polymorphism at codon 129, at least *in vitro* (Apetri et al., 2005). These studies provide a molecular rationale for the observed strain specificity of FFI and 178CJD.

Another hypothesis held forth was a role for the 129 codon polymorphism in modifying the interaction of the PrP with a receptor or protein present in thalamolimbic structures (Lugaresi et al., 1998). The picture is further complicated by the finding that even the 129 codon polymorphism on the nonmutated allele may exert an influence on the clinical phenotype; indeed, patients with a short disease course (less than 11 months) were found to be predominantly met/met homozygotes at the 129 *PRNP* codon, whereas a more prolonged disease was a feature in met/val heterozygote patients (Montagna et al., 1998). How this may happen remains unclear, especially when considering that the nonmutated allele does not contribute to PrPSc generation in FFI (Chen et al., 1997). Moreover, considerable variation in phenotypic expression has been reported even within 129 met/met homozygotes (Zarranz et al., 2005), suggesting that other genetic/environmental factors are at work in modifying the clinical expression of the 178 codon mutation.

SPORADIC FATAL INSOMNIA

In 1999, the first cases were reported of sporadic patients displaying the clinical and neuropathological features typical of FFI in the absence of a family history and of the specific D178N mutation that typifies the disease, or indeed of any other *PRNP* mutation (Mastrianni et al., 1999; Parchi et al., 1999). Such cases were termed sporadic fatal insomnia (SFI), and it was shown that type of PrPSc deposited in the brain (type 2) and amount and pattern of deposition (with a preferential deposition in the thalamus) were the same as those observed in FFI. Another characteristic of SFI is represented by the fact that all observed cases have been met/met homozygotes at codon 129 of *PRNP*. That the sleep abnormalities typical of FFI also characterize SFI was shown in a study by Scaravilli et al. (2000), who performed polysomnography in a patient with SFI. Remarkably, SFI could be transmitted to the experimental animal much in the same way as FFI (Mastrianni et al., 1999), and transmission experiments showed that both type of PrPSc and histopathological lesion profile were the same as FFI. This was considered further proof that the conformation of the protein itself codifies for its pathogenic properties.

FFI and SFI as model diseases for sleep regulation: the role of the thalamus

Correlation of the clinical features of FFI with brain *in vivo* metabolic and *post-mortem* neuropathological findings implicate the thalamus in the genesis of the peculiar abnormalities observed in FFI, specifically the wake–sleep alterations and autonomic changes.

Prominent abnormalities of the wake–sleep cycle, in particular the loss of sleep spindling and a marked reduction of SWS, and autonomic hyperactivity can be observed at stages of the disease when the only significant abnormalities in brain FDG-PET consist of hypometabolism of the thalami and the cingular cortex. Therefore it was contended that disruption in the thalamolimbic circuits represents the fundamental pathogenic mechanism underlying the sleep and autonomic alterations of FFI (Tinuper et al., 1989; Sforza et al., 1995; Lugaresi et al., 1998).

The mediodorsal and the anterior ventral thalamic nuclei represent the fulcrum of the pathological changes in FFI. Both of these nuclei have connections with forebrain limbic areas, and represent intermediate stations in the circuitry that connects the cortex with the subcortical hypothalamic and brainstem regions devoted to sleep and wake regulation. In FFI, the lesions in the thalamus would relieve some of these hypothalamic and brainstem structures of cortical control. Activation of these subcortical hypothalamic and brainstem regions would in turn lead to autonomic and motor hyperactivity, and inability to produce deep sleep. That sleep spindles and K-complexes disappear early in the course of FFI is indeed consistent with their thalamic origin in the reticular nucleus (Steriade, 2003). Even though pathology in the reticular nucleus is difficult to ascertain in FFI, it should be noted that the reticular nucleus sends efferents to the mediodorsal nucleus (Velayos et al., 1998), and moreover that lesions of the reticular nucleus itself have been shown to reduce the amount of EEG delta oscillations and to result in death of the experimental animal (Marini et al., 2000).

On the other hand, FFI as a model disease vindicates the early studies of Hess (1944), which implicated the thalamus in the pathogenetic mechanisms of sleep. Hess found that electrical stimulation of the medial thalamus induced sleep in the cat, and accordingly hypothesized that the medial thalamus exerts a trophotropic influence, in the sense of favoring those bodily functions devoted to energy restoration (whereas the lateral thalamus exerts ergotropic functions, i.e., aimed at energy expenditure). Hess's findings, confirmed by Parmeggiani (1964), were later disputed by Naquet et al. (1965), so that the thalamus was not considered to be involved in the regulation of sleep any more, and even disappeared from some textbooks of sleep physiology.

However, evidence for a role for the thalamus in deep sleep generation was also provided by Villablanca et al. (1972a, b), who demonstrated that thalamectomized cats (so-called “athalamic” cats) had severely reduced REM and NREM sleep; remarkably, these cats

had permanently absent sleep spindles that could not be evoked by thiopental administration, in a way reminiscent of patients with FFI (Tinuper et al., 1989). Marked reductions in REM and NREM sleep in the face of preserved sleep spindles were also observed in cats with intact thalamus but ablated cortex and striatum (so-called “diencephalic” cats). These studies bear, importantly, on the issue of FFI and sleep regulation, as FFI is characterized pathologically by atrophy and hypometabolism of the thalamus *plus* the limbic cortex, and accordingly it is to be expected that these combined lesions be attended by even more prominent abnormalities and loss of SWS. Intriguingly, and similar to the findings in FFI, both “diencephalic” and “athalamic” cats, although unable to sleep, still continued to display drowsiness. Ibotenic lesions in the mediodorsal thalamic nucleus reduced the amount of SWS in the cat, whereas comparable lesions in the anterior thalamic nucleus remained ineffective (Marini et al., 1988, 1989).

The evidence from experimental animal studies is also supplemented by clinical reports: bilateral stereotactic lesions of the thalamus in a parkinsonian patient were followed by persistent insomnia (Bricolo, 1967), and patients with bilateral paramedian lesions of the thalamus (because of vascular insult or calcifications) display loss of sleep spindles and deep sleep, and also abnormal autonomic functions in many ways resembling FFI (Guilleminault et al., 1993; Bassetti et al., 1996; Montagna et al., 2002). These patients pass most of the daytime in a state of “subwakefulness” (or presleep behavior), akin to stage 1 NREM sleep, reminiscent of FFI and of the “athalamic” and “diencephalic” cats of Villablanca.

Finally, a role for the thalamus in sleep, particularly in SWS generation, was provided by neuroimaging studies in humans, whereby synchronized sleep was attended by thalamic hypometabolism (Andersson et al., 1998) and SWS by lower metabolic rate in the thalamus and limbic and paralimbic areas (Braun et al., 1997). Cerebral blood flow covaries negatively with EEG delta activities, particularly in the thalamus, the orbitofrontal and cingular cortex, and the brainstem reticular formation (Hofle et al., 1997). It is indeed remarkable that the pattern of neural deactivation during physiological deep sleep in humans resembles the PET studies in FFI. Medial thalamic structures also represent centers of integration of autonomic activities. A role for the dorsomedial nucleus in the regulation of autonomic activities was demonstrated by studies with bicuculline injections, which caused hypertension and tachycardia through mechanisms implying the removal of GABAergic tonic inhibition (Stotz-Potter and Benarroch, 1998).

This whole body of observations demonstrates that the thalamus is a neural region important for the generation not only of sleep spindles, which signal the transition from light to deep sleep (the sleep “proper”), but of SWS itself. The thalamus acts in concert with the forebrain, especially limbic systems. Indeed, the mediodorsal nucleus is integrated within pathways coming from the hypothalamus and basal forebrain, with direct GABAergic interconnections (Gritti et al., 1998). The thalamic lesions in FFI probably introduce a diaschisis between the forebrain cortical regions and the hypothalamus and the brainstem areas governing sleep and autonomic functions (Lugaresi et al., 1998), resulting in a functional imbalance with loss of SWS and autonomic hyperactivity (Hess’s ergotropic activation). If we conceive of sleep as a kind of instinctive behavior (Moruzzi, 1969), the medial thalamus and mesial limbic cortex may be conceptualized as repositories of neural representations of motivational drives, including the need for sleep (Sewards and Sewards, 2003).

A question mark pertains, however, to the role played by atrophy of the inferior olives in the symptomatology of FFI. It has been hypothesized that tremor and myoclonus are clinical features possibly related to the olivary atrophy and attending serotonergic abnormalities (the inferior olives receive a substantial serotonergic innervation) (Welsh et al., 2002). Remarkably, however, the inferior olives project to the cerebellum, and cerebellectomy (or lesions of the superior cerebellar peduncle) in cats alters the wake–sleep cycle by specifically producing a significant increase in drowsiness episodes, and a shortening of wake and SWS, with lengthening of paradoxical sleep (Cunchillos and De Andres, 1982), features reminiscent of FFI. Therefore, a possible role for the inferior olivary atrophy (through the intermediation of the cerebellum?) in the pathogenesis of the sleep disturbances of FFI, specifically the increased subwakefulness state, cannot be discounted.

FFI and the concept of “agrypnia excitata”

FFI and SFI do not represent the only diseases characterized by loss of sleep, and autonomic and motor overactivation. Similar clinical features and polysomnographic findings have been reported in other clinical conditions unrelated to prion pathologies, such as *delirium tremens*, a syndrome of withdrawal from chronic alcohol and benzodiazepine or meprobamate chronic intoxication (Plazzi et al., 2002b), and *Morvan’s chorea*, which can be considered an autoimmune limbic encephalitis associated with peripheral neuro-myotonia (Liguori et al., 2001). Accordingly, the term “agrypnia excitata” (*agrypnia* meaning “chasing sleep

away” and referring to sleep loss of organic origin, and *excitata* referring to the motor and autonomic activation) was coined for patients who display prominent sleep alterations such as loss of SWS and oneiric behaviors, associated with hyperactivity in sympathetic functions and with motor overactivity not respecting circadian rhythmicity (Lugaresi and Provini, 2001). Based on the clinicopathological and brain metabolic correlations already found in FFI, agrypnia excitata was considered a dysfunction within the thalamolimbic circuits, and proposed as a useful concept in clinical neurology (Montagna and Lugaresi, 2002).

The concept of agrypnia excitata may help in everyday practice to define patients and try to localize the anatomical correlates of their clinical features. Moreover, it allows intriguing considerations on the physiopathology of sleep that bear not only on the conceptualization of the wake–sleep states, but also on the current scoring methods of sleep. FFI has been thus construed as a model disease for sleep physiopathology, akin to Von Economo’s clinicopathological studies of encephalitis lethargica which allowed him to appreciate the role of the hypothalamus in sleep regulation. A remarkable feature of agrypnia excitata is the fact that patients display a normal occurrence of drowsiness in the face of severely abnormal and sometimes completely absent SWS and REM sleep stages. Various defined as “subwakefulness”, “pseudo-hypersomnia”, or “presleep behavior” in patients affected with paramedian thalamic lesions and attributed to “de-arousal” (Guilleminault et al., 1993; Bassetti et al., 1996), these periods of drowsiness resemble polygraphically stage 1–2 NREM sleep and may represent the predominant state during daytime. In agrypnia excitata it is possible to have normal wake and light sleep stages (stage 1 NREM sleep), but sleep spindles (i.e., NREM sleep stage 2 and deeper sleep) and SWS cannot be generated. This calls for a different conceptualization of NREM sleep, and for a scoring system different from that currently in use (Rechtschaffen and Kales, 1968).

NREM sleep is currently considered a monotonic process, in which synchronization of EEG sleep waves occurs progressively from stage 1 and 2 (light sleep) down to deeper sleep stages characterized by delta EEG. Sleep spindles herald the transition from light to deep sleep stages. Such a view is controversial, however, as not all authors accept that light sleep be put together with SWS into a single physiological process. For instance, when behavioral monitoring (response to auditory bursts) was applied to the definition of the sleep stages and compared with EEG scoring, only in EEG stages 3, 4, and REM sleep could a significant distinction with wakefulness be achieved

(Dement and Kleitman, 1957; Ogilvie et al., 1989). This led Ogilvie et al. (1989) to separate a “sleep-onset period” corresponding to light sleep, and during which cognitive response is still possible.

The findings in FFI and agrypnia excitata concur with the view that light sleep phases up to the development of the sleep spindles should be separated from SWS, and that they should be conceptualized as a distinct vigilance state, whose relationship with relaxed vigilance and drowsiness should be reappraised (Montagna and Lugaresi, 2002). These light sleep phases are, moreover, identified cognitively by peculiar mental processes (mental imageries, absence of rational thought, spatial and temporal illusions) that led Critchley to recognize the specificity of, and to propose, the term “predormitum” for this particular state (Critchley, 1955). Moreover, the “predormitum” is the time for the occurrence of specific physiological (slow eye movements, hypnic jerks) and pathological (proprio-spinal myoclonus at the wake-sleep transition; Montagna et al., 1997) motor events, which also argue for the specificity of this vigilance state. Thus, following the concept of sleep as a kind of instinctive behavior (Moruzzi, 1969), the appetitive phase corresponding to “presleep behavior” or drowsiness/light sleep stage 1 NREM sleep is followed under appropriate conditions by the consummatory phase, corresponding to the SWS/REM sleep cycles.

The basic mechanisms and functions underlying the generation of these two different states of “sleep” must ultimately be different. As drowsiness is still possible with severe thalamic lesions, the mechanisms for generation of drowsiness or sleep onset must reside elsewhere. There is evidence that a dissociation of rest versus true sleep can be effected in the experimental animal (Villablanca, 1966), and rest behavior but not true sleep is found in chronic medullary and midpontine cats (Siegel et al., 1986). Based on lesion experiments, Villablanca (Villablanca, 1966; Villablanca et al., 2001) concluded that sleep and wakefulness are controlled by dual independent mechanisms, with “brain sleep (i.e., true sleep, SWS) and wakefulness” originating in the rostral brain, and “body sleep (i.e., rest, drowsiness) and wakefulness” originating in caudal brainstem structures. These considerations are also consistent with a phylogenic view of sleep as a process emerging from the rest-wake rhythmicity displayed by lower (poikilothermic) animals that cycle between rest and activity, whereas true sleep (both SWS and REM sleep) is found only in homeotherms, in agreement with the “presleep theory” of the ontogenetic development of sleep, whereby both REM sleep and SWS emerge from a common “dissociated” state in neonates (Frank and Heller, 1997).

In accordance with these views, and with the clinical and laboratory findings derived from agrypnia excitata, Lugaresi et al. (2004) proposed that drowsiness/light stage 1 NREM sleep represents an independent vigilance state with autonomous mechanisms and functions, *on a par* with wakefulness, whereas deep (slow wave) sleep and REM sleep are comparable to the “rest” state of the poikilothermic animals. They also suggested a caudorostral neural organization of these three sleep stages: “rest”/drowsiness being organized at a caudal brainstem (reticular?) level, REM sleep at a pontine level, and SWS at a more rostral, thalamo-lymbic level.

Prions and sleep

The function(s) of the prion protein remains still unknown. Its relatively well preserved structure throughout evolution seems to indicate an essential role in cell biology. Prions have neuroprotective properties (Chiarini et al., 2002) and have been implicated in cellular adhesion and cell signaling (Martins and Brentani, 2002). As prions bind copper and they are taken up actively into caveolae, acting as a sink for copper, they could have a role in copper metabolism (Brown and Sassoon, 2002), which would translate into a protective action from oxidative stress. The abundant expression of PrPC in the hippocampus has been taken to indicate an essential role of prions in memory formation (Tompa and Friedrich, 1998). However, nitrergic mechanisms may play a role in the pathophysiology of the prion diseases (Velayos and Alfageme, 1999) and the presence of the cellular PrP isoform in a subset of GABAergic neurons containing calcium binding proteins suggests that PrPC plays a role in the metabolism of calcium (Moleres and Velayos, 2005).

Gleaned from the abnormal findings in FFI, a role proposed for the prion protein was sleep regulation (Tobler et al., 1997). Mice devoid of PrP (knockout mice, 129/SV “null”) display an alteration in both circadian activity rhythms and patterns (Tobler et al., 1996). Moreover, when these null mice are studied for sleep abnormalities, they display sleep fragmentation with increased numbers of waking episodes and abnormal (faster but lesser) buildup of slow-wave activity at the wake to NREM sleep transitions. These mice also express a larger and more prolonged increase in slow-wave activity when recovering from sleep deprivation (Tobler et al., 1997).

On the basis of these findings, a role for the prion protein in promoting sleep continuity (Tobler et al., 1997) and more generally in sleep regulation (Huber et al., 1999) was proposed. In later studies, however, a conclusion was reached that differences between

the null and control mice could be better explained by a different need for recovery after sleep deprivation expressed by the occipital brain areas; indeed the larger increase of slow-wave activity after sleep deprivation was restricted to the occipital regions, and these differences appeared after the waking–NREM sleep transitions, making it unlikely that PrP is involved in the mechanisms enabling the transition to sleep (Huber et al., 2002). Thus, there is only equivocal experimental evidence for a role of the prion protein in sleep regulation. The clinical evidence, although forthcoming, remains also circumstantial.

Several lines of evidence indicate that animals infected with prions exhibit sleep abnormalities, often in the early stages of the disease. Early disturbances of consciousness were noted in squirrel monkeys infected with CJD agents (Cathala et al., 1981). After scrapie inoculation, rats displayed spindle-shaped bursts of diphasic spikes during quiet wakefulness, and changes in the sleep–wakefulness cycle, characterized by decreased active wakefulness, increased quiet wakefulness, and diminished SWS. These EEG disturbances clearly preceded the onset of the clinical signs (Bassant et al., 1984). Twenty months after intracerebral inoculation of a CJD agent, cats developed unusual sleep, especially REM sleep, abnormalities (Gourmelon et al., 1987). Two strains of transmissible mink encephalopathy (TME) are known, called hyper (HY) and drowsy (DY). After passage into hamsters, although HY hamsters manifest hyperesthesia and cerebellar ataxia, lethargy and drowsiness are found in DY hamsters (Bessen and Marsh, 1992). These different clinical features in mink encephalopathy are related to different scrapie strains with distinct PrP properties (Bessen and Marsh, 1994), in a way reminiscent of the distinctive features of FFI versus 178CJD, and confirm that strain diversity encoded in different structural properties of the prion protein specifies the clinical differences. Also, monitoring of circadian activity revealed early abnormalities, most pronounced during the nocturnal active phase in mice with bovine spongiform encephalopathy and scrapie (Dell’Omo et al., 2002).

Similar findings are seen in some human prion encephalopathies. Generally, the changes in sleep organization occur with the beginning of the clinical phase (Court and Bert, 1995) and precocious loss of sleep was documented by serial polysomnography in a few patients with sporadic CJD (Vitrey et al., 1971; Terzano et al., 1995). These findings are not, however, universal in the human prion encephalopathies: studies performed in GSS have demonstrated a completely normal structure of sleep and circadian organization (Pierangeli et al., 2004), casting doubt on the proposal

that the prion protein itself is responsible for the abnormal sleep–wake regulation observed in FFI. These negative findings are more in agreement with the pathological location of changes in the thalamolimbic areas as responsible for the sleep–wake abnormalities, and this contention is also supported by those cases of human prion encephalopathies different from FFI in which prominent abnormalities of the sleep–wake and other circadian rhythms were observed, as in many of these cases pathology included relevant changes in thalamic regions (Chapman et al., 1996; Taratuto et al., 2002). Polysomnographic studies performed in presymptomatic carriers of the FFI *PRNP* mutation also support such an explicatory hypothesis, as no significant differences were found in the absolute and relative power of the alpha, sigma, theta and delta EEG bands in presymptomatic FFI carriers, and sleep macrostructure was comparable to that in noncarriers (Ferrillo et al., 2001). Moreover, in a follow-up polysomnographic and PET study, loss of the sigma band (corresponding to spindle activity) on spectral EEG analysis was found to be associated with thalamic hypometabolism 13 months before the onset of the clinical symptoms of FFI (Cortelli et al., 2006), suggesting that the earliest sleep abnormalities in FFI, i.e., loss of sleep spindles, occur concomitantly with the metabolic changes in the thalamus. The role of the 129 codon polymorphism in FFI was instead underlined by a study of spectral EEG characteristics comparing 129 met/val against 129 met/met carriers: spindle frequency band power and delta/spindle activity ratio were found correlated with 129 *PRNP* codon polymorphism, which was especially abnormal in the met/met homozygotes (Plazzi et al., 2002a). A role, however, for the codon 129 *PRNP* polymorphism for insomnia complaints in the general population was not confirmed in a genetic association study (Pedrazzoli et al., 2002).

CONCLUSIONS

FFI has offered important insights into the pathophysiology of the prion diseases, and at the same time has been proposed as a model disease for a role of the thalamus and thalamolimbic circuits in the regulation of sleep and of the autonomic nervous system. Comparison of the clinical and pathological features between FFI and 178CJD allowed the first recognition of a novel mechanism for genetic heterogeneity, related to the influence of an intragenic (codon 129) polymorphism; moreover, FFI has been instrumental in the recognition of the existence of different prion strains (e.g., type 1 (21 kDa) and type 2 (19 kDa) PrP^{Sc}) and, following the transmission experiments, has helped to confirm the “protein only” theory of prion infectivity, whereby

the phenotypic characteristics of the disease are enciphered in the protein conformational structure and are independent of any DNA transmitted by the donor of the inoculum. This is an important new paradigm in the biological sciences.

In the study of sleep and the thalamus, clinicopathological correlations in FFI implicated the anterior and medial thalamus and their limbic connections in the generation of SWS. As a consequence, the concept of *agrypnia excitata* was proposed as a useful concept in clinical neurology and with relevant consequences for sleep physiology. The existence of normal light sleep *vis á vis* with loss or even absence of SWS argues for intrinsically different basic mechanisms underlying these two types of sleep, and moreover suggests that they are organized in different brain regions and subserve different functions. Accordingly, it has been proposed that light sleep/drowsiness represents an autonomous state of being, on a par with wake, deep, and REM sleep, and that a reappraisal of the concept of NREM sleep and its scoring methods is necessary.

REFERENCES

- Almer G, Hainfellner JA, Brucke T et al. (1999). Fatal familial insomnia: a new Austrian family. *Brain* 122: 5–16.
- Andersson JL, Onoe H, Hetta J et al. (1998). Brain networks affected by synchronized sleep visualized by positron emission tomography. *J Cereb Blood Flow Metab* 18: 701–715.
- Apetri AC, Vanik DL, Surewicz WK (2005). Polymorphism at residue 129 modulates conformational conversion of the D178N variant of human prion protein 90–231. *Biochemistry* 44: 15880–15888.
- Avoni P, Cortelli P, Montagna P et al. (1991). Circadian hormonal rhythms in two new cases of fatal familial insomnia. *Acta Neurol* 13: 574–576.
- Bar KJ, Hager F, Nenadic I et al. (2002). Serial positron emission tomographic findings in an atypical presentation of fatal familial insomnia. *Arch Neurol* 59: 1815–1818.
- Bassant MH, Cathala F, Court L et al. (1984). Experimental scrapie in rats: first electrophysiological observations. *Electroencephalogr Clin Neurophysiol* 57: 541–547.
- Bassetti C, Mathis J, Gugger M et al. (1996). Hypersomnia following paramedian thalamic stroke: a report of 12 patients. *Ann Neurol* 39: 471–480.
- Bessen RA, Marsh RF (1992). Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. *J Gen Virol* 73: 329–334.
- Bessen RA, Marsh RF (1994). Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J Virol* 68: 7859–7868.
- Bosque PJ, Vnencak-Jones CL, Johnson MD et al. (1992). A PrP gene codon 178 base substitution and a 24-bp interstitial deletion in familial Creutzfeldt–Jakob disease. *Neurology* 42: 1864–1870.
- Brandner S, Raeber A, Sailer A et al. (1996). Normal host prion protein (PrPC) is required for scrapie spread within the central nervous system. *Proc Natl Acad Sci U S A* 93: 13148–13151.
- Braun AR, Balkin TJ, Wesenten NJ et al. (1997). Regional cerebral blood flow throughout the sleep–wake cycle. An H2(15)O PET study. *Brain* 120: 1173–1197.
- Bricolo A (1967). Insomnia after bilateral stereotactic thalamotomy in man. *J Neurol Neurosurg Psychiatry* 30: 154–158.
- Brown DR, Sassoone J (2002). Copper-dependent functions for the prion protein. *Mol Biotechnol* 22: 165–178.
- Cathala F, Court L, Breton P et al. (1981). Creutzfeldt–Jakob disease in the squirrel monkeys. *Rev Neurol (Paris)* 137: 785–805.
- Chapman J, Arlazoroff A, Goldfarb LG et al. (1996). Fatal insomnia in a case of familial Creutzfeldt–Jakob disease with the codon 200(Lys) mutation. *Neurology* 46: 758–761.
- Chen SG, Parchi P, Brown P et al. (1997). Allelic origin of the abnormal prion protein isoform in familial prion diseases. *Nat Med* 3: 1009–1015.
- Chiarini LB, Freitas AR, Zanata SM et al. (2002). Cellular prion protein transduces neuroprotective signals. *EMBO J* 21: 3317–3326.
- Choi SI, Ju WK, Choi EK et al. (1998). Mitochondrial dysfunction induced by oxidative stress in the brains of hamsters infected with the 263 K scrapie agent. *Acta Neuropathol (Berl)* 96: 279–286.
- Collinge J (2001). Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 24: 519–550.
- Collinge J, Palmer MS, Sidle KC et al. (1995). Transmission of fatal familial insomnia to laboratory animals. *Lancet* 346: 569–570.
- Collins SJ, Lawson VA, Masters CL (2004). Transmissible spongiform encephalopathies. *Lancet* 363: 51–61.
- Cortelli P, Parchi P, Contin M et al. (1991). Cardiovascular dysautonomia in fatal familial insomnia. *Clin Auton Res* 1: 15–21.
- Cortelli P, Perani D, Parchi P et al. (1997). Cerebral metabolism in fatal familial insomnia: relation to duration, neuropathology, and distribution of protease-resistant prion protein. *Neurology* 49: 126–133.
- Cortelli P, Polinsky R, Montagna P et al. (2001). Alteration of the serotonergic system in fatal familial insomnia. *Ann Neurol* 50: 421–422.
- Cortelli P, Perani D, Montagna P et al. (2006). Pre-symptomatic diagnosis in fatal familial insomnia: serial neurophysiological and [18F] FDG PET studies. *Brain* 129: 668–675.
- Court L, Bert J (1995). Electrophysiology of transmissible encephalopathies. *Pathol Biol (Paris)* 43: 25–42.
- Critchley M (1955). The pre-dormitum. *Rev Neurol (Paris)* 93: 101–106.
- Cunchillos JD, De Andres I (1982). Participation of the cerebellum in the regulation of the sleep–wakefulness cycle. Results in cerebellectomized cats. *Electroencephalogr Clin Neurophysiol* 53: 549–558.

- Dauvilliers Y, Cervenka K, Carlander B et al. (2004). Dissociation in circadian rhythms in a pseudohypersomnia form of fatal familial insomnia. *Neurology* 63: 2416–2418.
- DeArmond SJ, Mobley WC, DeMott DL et al. (1998). Changes in the localization of brain prion proteins during scrapie infection. *Neurology* 50: 1271–1280.
- Dell'Omo G, Vannoni E, Vyssotski AL et al. (2002). Early behavioural changes in mice infected with BSE and scrapie: automated home cage monitoring reveals prion strain differences. *Eur J Neurosci* 16: 735–742.
- Dement W, Kleitman N (1957). Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. *Electroencephalogr Clin Neurophysiol* 9: 673–690.
- Dorandeu A, Wingertsmann L, Chretien F et al. (1998). Neuronal apoptosis in fatal familial insomnia. *Brain Pathol* 8: 531–537.
- Ferrillo F, Plazzi G, Nobili L et al. (2001). Absence of sleep EEG markers in fatal familial insomnia healthy carriers: a spectral analysis study. *Clin Neurophysiol* 112: 1888–1892.
- Florio T, Thellung S, Amico C et al. (1998). Prion protein fragment 106–126 induces apoptotic cell death and impairment of L-type voltage-sensitive calcium channel activity in the GH3 cell line. *J Neurosci Res* 54: 341–352.
- Frank MG, Heller HC (1997). Development of REM and slow wave sleep in the rat. *Am J Physiol* 272: 1792–1799.
- Gallassi R, Morreale A, Montagna P et al. (1992). "Fatal familial insomnia": neuropsychological study of a disease with thalamic degeneration. *Cortex* 28: 175–187.
- Gallassi R, Morreale A, Montagna P et al. (1996). Fatal familial insomnia: behavioral and cognitive features. *Neurology* 46: 935–939.
- Goldfarb LG, Haltia M, Brown P et al. (1991). New mutation in scrapie amyloid precursor gene (at codon 178) in Finnish Creutzfeldt–Jakob kindred. *Lancet* 337: 425.
- Goldfarb LG, Petersen RB, Tabaton M et al. (1992). Fatal familial insomnia and familial Creutzfeldt–Jakob disease: disease phenotype determined by a DNA polymorphism. *Science* 258: 806–808.
- Gourmelon P, Amyx HL, Baron H et al. (1987). Sleep abnormalities with REM disorder in experimental Creutzfeldt–Jakob disease in cats: a new pathological feature. *Brain Res* 411: 391–396.
- Gritti I, Mariotti M, Mancina M (1998). GABAergic and cholinergic basal forebrain and preoptic–anterior hypothalamic projections to the mediodorsal nucleus of the thalamus in the cat. *Neuroscience* 85: 149–178.
- Guentchev M, Wanschitz J, Voigtlander T et al. (1999). Selective neuronal vulnerability in human prion diseases: fatal familial insomnia differs from other types of prion diseases. *Am J Pathol* 155: 1453–1457.
- Guilleminault C, Quera-Salva MA, Goldberg MP (1993). Pseudohypersomnia and pre-sleep behaviour with bilateral paramedian thalamic lesions. *Brain* 116: 1549–1563.
- Harder A, Jendroska K, Kreuz F et al. (1999). Novel twelve generation kindred of fatal familial insomnia from Germany representing the entire spectrum of disease expression. *Am J Med Genet* 87: 311–316.
- Hauw JJ, Szudovitch V, Laplanche JL et al. (2000). Neuro-pathologic variants of sporadic Creutzfeldt–Jakob disease and codon 129 of PrP gene. *Neurology* 54: 1641–1646.
- Hess WR (1944). Das Schlafsyndrom als Folge diencephaler Reizung. *Helv Physiol Pharmacol Acta* 2: 305–344.
- Hofle N, Paus T, Reutens D et al. (1997). Regional cerebral blood flow changes as a function of delta and spindle activity during slow wave sleep in humans. *J Neurosci* 17: 4800–4808.
- Huber R, Deboer T, Tobler I (1999). Prion protein: a role in sleep regulation? *J Sleep Res* 8 (Suppl 1): 30–36.
- Huber R, Deboer T, Tobler I (2002). Sleep deprivation in prion protein deficient mice and control mice: genotype dependent regional rebound. *Neuroreport* 13: 1–4.
- Johnson MD, Vnencak-Jones CL, McLean MJ (1998). Fatal familial insomnia: clinical and pathologic heterogeneity in genetic half brothers. *Neurology* 51: 1715–1717.
- Kloppel S, Pirker W, Brucke T et al. (2002). Beta-CIT SPECT demonstrates reduced availability of serotonin transporters in patients with fatal familial insomnia. *J Neural Transm* 109: 1105–1110.
- Kovács GG, Trabattoni G, Hainfellner JA et al. (2002). Mutations of the prion protein gene phenotypic spectrum. *J Neurol* 249: 1567–1582.
- Liguori R, Vincent A, Clover L et al. (2001). Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage gated potassium channels. *Brain* 124: 2417–2426.
- Lugaresi E, Provini F (2001). Agrypnia excitata: clinical features and pathophysiological implications. *Sleep Med Rev* 5: 313–322.
- Lugaresi E, Medori R, Montagna P et al. (1986). Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. *N Engl J Med* 315: 997–1003.
- Lugaresi E, Tobler I, Gambetti P et al. (1998). The pathophysiology of fatal familial insomnia. *Brain Pathol* 8: 521–526.
- Lugaresi E, Provini F, Montagna P (2004). The neuroanatomy of sleep. Considerations on the role of the thalamus in sleep and a proposal for a caudorostral organization. *Eur J Anat* 8: 85–93.
- Macchi G, Rossi G, Abbamondi AL et al. (1997). Diffuse thalamic degeneration in fatal familial insomnia: a morphometric study. *Brain Res* 771: 154–158.
- Ma J, Lindquist S (2002). Conversion of PrP to a self-perpetuating PrP^{Sc}-like conformation in the cytosol. *Science* 298: 1785–1788.
- Manetto V, Medori R, Cortelli P et al. (1992). Fatal familial insomnia: clinical and pathologic study of five new cases. *Neurology* 42: 312–319.
- Marini G, Imeri L, Mancina M (1988). Changes in sleep–waking cycle induced by lesions of medialis dorsalis thalamic nuclei in the cat. *Neurosci Lett* 85: 223–227.
- Marini G, Gritti I, Mancina M (1989). Changes in EEG spindle activity induced by ibotenic acid lesions of medialis dorsalis thalamic nuclei in the cat. *Brain Res* 500: 395–399.
- Marini G, Ceccarelli P, Mancina M (2000). Effects of bilateral microinjections of ibotenic acid in the thalamic reticular

- nucleus on delta oscillations and sleep in freely-moving rats. *J Sleep Res* 9: 359–366.
- Martinez-Rodriguez JE, Sanchez-Valle R, Saiz A et al. (2003). Normal hypocretin-1 levels in the cerebrospinal fluid of patients with fatal familial insomnia. *Sleep* 26: 1068.
- Martins VR, Brentani RR (2002). The biology of the cellular prion protein. *Neurochem Int* 41: 353–355.
- Mastrianni JA, Nixon R, Layzer R et al. (1999). Prion protein conformation in a patient with sporadic fatal insomnia. *N Engl J Med* 340: 1630–1638.
- Masullo C, Macchi G (2001). Does PRNP gene control the clinical and pathological phenotype of human spongiform transmissible encephalopathies? *Clin Neuropathol* 20: 19–25.
- McLean CA, Storey E, Gardner RJ et al. (1997). Diverse clinicopathologic phenotypes in an FFI Australian kindred. *Neurology* 49: 552–558.
- Medori R, Tritschler HJ, LeBlanc A et al. (1992a). Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N Engl J Med* 326: 444–449.
- Medori R, Montagna P, Tritschler HJ et al. (1992b). Fatal familial insomnia: a second kindred with mutation of prion protein gene at codon 178. *Neurology* 42: 669–670.
- Moleres FJ, Velayos JL (2005). Expression of PrP(C) in the rat brain and characterization of a subset of cortical neurons. *Brain Res* 1056: 10–21.
- Monari L, Chen SG, Brown P et al. (1994). Fatal familial insomnia and familial Creutzfeldt–Jacob disease: different prion proteins determined by a DNA polymorphism. *Proc Natl Acad Sci U S A* 91: 2839–2842.
- Montagna P, Lugaresi E (2002). Agrypnia excitata: a generalized overactivity syndrome and a useful concept in the neurophysiopathology of sleep. *Clin Neurophysiol* 113: 552–560.
- Montagna P, Cortelli P, Gambetti P et al. (1995). Fatal familial insomnia: sleep, neuroendocrine and vegetative alterations. *Adv Neuroimmunol* 5: 13–21.
- Montagna P, Provini F, Plazzi G et al. (1997). Propriospinal myoclonus upon relaxation and drowsiness: a cause of severe insomnia. *Mov Disord* 12: 66–72.
- Montagna P, Cortelli P, Avoni P et al. (1998). Clinical features of fatal familial insomnia: phenotypic variability in relation to a polymorphism at codon 129 of the prion protein gene. *Brain Pathol* 8: 515–520.
- Montagna P, Provini F, Plazzi G et al. (2002). Bilateral paramedian thalamic syndrome: abnormal circadian wake–sleep and autonomic functions. *J Neurol Neurosurg Psychiatry* 73: 772–774.
- Moruzzi G (1969). Sleep and instinctive behavior. *Arch Ital Biol* 107: 175–216.
- Nagayama M, Shinohara Y, Furukawa H et al. (1996). Fatal familial insomnia with a mutation at codon 178 of the prion protein gene: first report from Japan. *Neurology* 47: 1313–1316.
- Naquet R, Denavit M, Lanoir J et al. (1965). Alterations transitoires ou définitives des zones d'encéphaliques chez le chat. In: M Jouvett (Ed.), *Aspects Anatomico-fonctionnelles de la Physiologie du Sommeil*. Editions du Centre Nationale de la Recherche Scientifique, Paris, pp. 107–131.
- Ogilvie RD, Wilkinson RT, Allison S (1989). The detection of sleep onset: behavioral, physiological, and subjective convergence. *Sleep* 12: 458–474.
- Pan KM, Baldwin M, Nguyen J et al. (1993). Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci U S A* 90: 10962–10966.
- Parchi P, Castellani R, Cortelli P et al. (1995). Regional distribution of protease-resistant prion protein in fatal familial insomnia. *Ann Neurol* 38: 21–29.
- Parchi P, Petersen RB, Chen SG et al. (1998). Molecular pathology of fatal familial insomnia. *Brain Pathol* 8: 539–548.
- Parchi P, Capellari S, Chin S et al. (1999). A subtype of sporadic prion disease mimicking fatal familial insomnia. *Neurology* 52: 1757–1763.
- Parmeggiani PL (1964). A study on the central representation of sleep behaviour. *Prog Brain Res* 6: 180–190.
- Pedrazzoli M, Ling L, Young TB et al. (2002). Effect of the prion 129 polymorphism on nocturnal sleep and insomnia complaints: a population-based study. *J Sleep Res* 11: 357–358.
- Perani D, Cortelli P, Lucignani G et al. (1993). [18F]FDG PET in fatal familial insomnia: the functional effects of thalamic lesions. *Neurology* 43: 2565–2569.
- Petersen RB, Tabaton M, Berg L et al. (1992). Analysis of the prion protein gene in thalamic dementia. *Neurology* 42: 1859–1863.
- Petersen RB, Goldfarb LG, Tabaton M et al. (1994). A novel mechanism of phenotypic heterogeneity demonstrated by the effect of a polymorphism on a pathogenic mutation in the PRNP (prion protein gene). *Mol Neurobiol* 8: 99–103.
- Petersen RB, Parchi P, Richardson SL et al. (1996). Effect of the D178N mutation and the codon 129 polymorphism on the metabolism of the prion protein. *J Biol Chem* 271: 12661–12668.
- Pierangeli G, Bono F, Aguglia U et al. (2004). Normal sleep–wake and circadian rhythms in a case of Gerstmann–Straussler–Sheinker (GSS) disease. *Clin Auton Res* 14: 39–41.
- Plazzi G, Schutz Y, Cortelli P et al. (1997). Motor overactivity and loss of motor circadian rhythm in fatal familial insomnia: an actigraphic study. *Sleep* 20: 739–742.
- Plazzi G, Montagna P, Beelke M et al. (2002a). Does the prion protein gene 129 codon polymorphism influence sleep? Evidence from a fatal familial insomnia kindred. *Clin Neurophysiol* 113: 1948–1953.
- Plazzi G, Montagna P, Meletti S et al. (2002b). Polysomnographic study of sleeplessness and oneiricisms in the alcohol withdrawal syndrome. *Sleep Med* 3: 279–282.
- Portaluppi F, Cortelli P, Avoni P et al. (1994a). Diurnal blood pressure variation and hormonal correlates in fatal familial insomnia. *Hypertension* 23: 569–576.
- Portaluppi F, Cortelli P, Avoni P et al. (1994b). Progressive disruption of the circadian rhythm of melatonin in fatal familial insomnia. *J Clin Endocrinol Metab* 78: 1075–1078.

- Portaluppi F, Cortelli P, Avoni P et al. (1995). Dissociated 24-hour patterns of somatotropin and prolactin in fatal familial insomnia. *Neuroendocrinology* 61: 731–737.
- Prusiner SB (1987). Prions and neurodegenerative diseases. *N Engl J Med* 317: 1571–1581.
- Puoti G, Rossi G, Giaccone G et al. (2000). Polymorphism at codon 129 of PRNP affects the phenotypic expression of Creutzfeldt–Jakob disease linked to E200K mutation. *Ann Neurol* 48: 269–270.
- Rechtschaffen A, Kales A (1968). *A Manual of Standardized Terminology, Techniques and Scoring Techniques for Sleep Stages of Human Subjects*. Brain Research Institute, Los Angeles.
- Roberts GW, Clinton J (1992). Prion disease: the spectrum of pathology and diagnostic considerations. In: SB Prusiner, J Collinge, J Powell et al. (Eds.), *Prion Diseases of Humans and Animals*. Ellis Horwood, New York, pp. 215–240.
- Rossi G, Macchi G, Porro M et al. (1998). Fatal familial insomnia: genetic, neuropathologic, and biochemical study of a patient from a new Italian kindred. *Neurology* 50: 688–692.
- Scaravilli F, Cordery RJ, Kretschmar H et al. (2000). Sporadic fatal insomnia: a case study. *Ann Neurol* 48: 665–668.
- Sewards TV, Sewards MA (2003). Representations of motivational drives in mesial cortex, medial thalamus, hypothalamus and midbrain. *Brain Res Bull* 61: 25–49.
- Sforza E, Montagna P, Tinuper P et al. (1995). Sleep–wake cycle abnormalities in fatal familial insomnia: evidence of the role of the thalamus in sleep regulation. *Electroencephalogr Clin Neurophysiol* 94: 398–405.
- Shamsir MS, Dalby AR (2005). One gene, two diseases and three conformations: molecular dynamics simulations of mutants of human prion protein at room temperature and elevated temperatures. *Proteins* 59: 275–290.
- Siegel JM, Tomaszewski KS, Nienhuis R (1986). Behavioral states in the chronic medullary and midpontine cat. *Electroencephalogr Clin Neurophysiol* 63: 274–288.
- Steriade M (2003). The corticothalamic system in sleep. *Front Biosci* 8: 878–899.
- Stotz-Potter E, Benarroch E (1998). Removal of GABAergic inhibition in the mediodorsal nucleus of the rat thalamus leads to increases in heart rate and blood pressure. *Neurosci Lett* 247: 127–130.
- Taberner C, Polo JM, Sevillano MD et al. (2000). Fatal familial insomnia: clinical, neuropathological, and genetic description of a Spanish family. *J Neurol Neurosurg Psychiatry* 68: 774–777.
- Taniwaki Y, Hara H, Doh-Ura K et al. (2000). Familial Creutzfeldt–Jakob disease with D178N-129M mutation of PRNP presenting as cerebellar ataxia without insomnia. *J Neurol Neurosurg Psychiatry* 68: 388.
- Taratuto AL, Piccardo P, Reich EG et al. (2002). Insomnia associated with thalamic involvement in E200K Creutzfeldt–Jakob disease. *Neurology* 58: 362–367.
- Tateishi J, Brown P, Kitamoto T et al. (1995). First experimental transmission of fatal familial insomnia. *Nature* 376: 434–435.
- Telling GC, Parchi P, DeArmond SJ et al. (1996). Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* 274: 2079–2082.
- Terzano MG, Parrino L, Pietrini V et al. (1995). Precocious loss of physiological sleep in a case of Creutzfeldt Jakob disease: a serial polygraphic study. *Sleep* 18: 849–858.
- Tinuper P, Montagna P, Medori R et al. (1989). The thalamus participates in the regulation of the sleep–waking cycle: a clinico-pathological study in fatal familial thalamic degeneration. *Electroencephalogr Clin Neurophysiol* 73: 117–123.
- Tobler I, Gaus SE, Deboer T et al. (1996). Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature* 380: 639–642.
- Tobler I, Deboer T, Fischer M (1997). Sleep and sleep regulation in normal and prion protein-deficient mice. *J Neurosci* 17: 1869–1879.
- Tomba P, Friedrich P (1998). Prion proteins as memory molecules: an hypothesis. *Neuroscience* 86: 1037–1043.
- Velayos JL, Alfageme F (1999). Forebrain and brainstem perivascular neurons projecting to the thalamus (an anatomical explanation of the pathophysiology of fatal familial insomnia). *Eur J Anat* 3: 87–92.
- Velayos JL, Oliva M, Alfageme F (1998). Afferent projections to the mediodorsal and anterior thalamic nuclei in the cat: anatomical–clinical correlations. *Brain Pathol* 8: 549–552.
- Villablanca J (1966). Behavioural and polygraphic study of ‘sleep’ and ‘wakefulness’ in chronic decerebrate cats. *Electroencephalogr Clin Neurophysiol* 219: 562–577.
- Villablanca J, Salinas-Zeballos ME (1972a). Sleep–wakefulness EEG and behavioral studies of chronic cats without the thalamus: the ‘athalamic’ cat. *Arch Ital Biol* 110: 383–411.
- Villablanca J, Marcus R (1972b). Sleep–wakefulness EEG and behavioral studies of chronic cats without neocortex and striatum: the ‘diencephalic’ cat. *Arch Ital Biol* 110: 348–382.
- Villablanca J, De Andr s I, Olmstead CE (2001). Sleep–waking states develop independently in the isolated forebrain and brain stem following early postnatal midbrain transection in cats. *Neuroscience* 106: 717–731.
- Vitrey M, Huguet P, Samson-Dollfus D (1971). Two sleep records obtained during the course of Jakob Creutzfeldt disease. *Electroencephalogr Clin Neurophysiol* 30: 253–254.
- Wanschitz J, Kloppel S, Jarius C et al. (2000). Alteration of the serotonergic nervous system in fatal familial insomnia. *Ann Neurol* 48: 788–791.
- Welsh JP, Placantonakis DG, Warsetsky SI et al. (2002). The serotonin hypothesis of myoclonus from the perspective of neuronal rhythmicity. *Adv Neurol* 89: 307–329.
- Zarranz JJ, Dagon A, Atares B et al. (2005). Phenotypic variability in familial prion diseases due to the D178N mutation. *J Neurol Neurosurg Psychiatry* 76: 1491–1496.
- Zerr I, Bodemer M, Gefeller O et al. (1998a). Detection of 14–3–3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt–Jakob disease. *Ann Neurol* 43: 32–40.
- Zerr I, Giese A, Windl O et al. (1998b). Phenotypic variability in fatal familial insomnia (D178N-129M) genotype. *Neurology* 51: 1398–1405.