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CLINICAL REVIEW

Fatal familial insomnia: a model disease in sleep physiopathology

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Agrypnia;
NREM sleep;
REM sleep;
Slow-wave sleep

Summary Fatal Familial Insomnia (FFI) is characterized by loss of sleep, oneiric stupor with autonomic/motor hyperactivity and somato-motor abnormalities (pyramidal signs, myoclonus, dysarthria/dysphagia, ataxia). Positon emission tomography (PET) disclosed thalamic hypometabolism and milder involvement of the cortex; neuropathology severe neuronal loss in the thalamic nuclei variably affecting the caudate, gyrus cinguli and fronto-temporal cortices. Genetic analysis disclosed a mutation in the *PRNP* gene and FFI was transmitted to experimental animals, thus classifying FFI within the prion diseases. Rare Sporadic Fatal Insomnia (SFI) cases occur without *PRNP* mutation but with features similar to FFI. FFI represents a model disease for the study of sleep-wake regulation: (I) the profound thalamic hypometabolism/atrophy associated with lack of sleep spindles and delta sleep implicate the thalamus in the origin of slow wave sleep (SWS); (II) loss of SWS is associated with marked autonomic and motor hyperactivity; termed 'agrypnia excitata', this association has been proposed as a useful clinical concept representative of thalamo-limbic dysfunction; (III) lack of SWS occurs with substantial preservation of stage 1 NREM sleep, implying that the latter has mechanisms different from SWS and unaffected by thalamic atrophy; accordingly, conflating stage 1 NREM with SWS into NREM sleep is inappropriate.

Introduction

Fatal Familial Insomnia (FFI) is a rare disease which belongs within the Transmissible Spongiform Encephalopathies (TSE), characterized by neurodegenerative changes due to the toxic effects of an abnormal constitutively expressed protein, the prion protein (PrP). TSE are both sporadic and hereditary. However, their characteristic feature is their infectivity, i.e. the possibility of transmission to humans and experimental animals. How TSE infectivity is accomplished remained for a long time a scientific mystery. In a significant reversal of the long standing 'nucleic acid only' paradigm of infectious diseases, most authors now accept that TSE infectivity resides with the PrP itself, related to conformational changes in the protein which are transmitted to the infected animal without the help of genetic information by the host.[1] Prions, the particles responsible for infectivity, are devoid of

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Nomenclature

Agrypnia	complete loss of sleep
BP	blood pressure
CJD	Creutzfeldt–Jakob Disease
FFI	Fatal Familial Insomnia
HR	heart rate
GSS	Gerstmann–Straussler–Scheinker disease
MRI	magnetic resonance imaging
NREM	non-rapid-eye movement
PrPC	cellular prion protein
PrPres	proteinase resistant prion protein
PrPSc	scrapie prion protein
RCBF	regional cerebral blood flow
REM	rapid eye movements
SFI	Sporadic Fatal Insomnia
SWS	slow wave sleep
T°	body core temperature
TSE	transmissible spongiform encephalopathies

nucleic acid and composed of a modified protein called PrPScrapie (PrPSc) characterized, amyloid-like, by a high β-sheet composition. PrPSc derives from a normal cellular protein PrP, constitutively expressed in the brain and called PrPCellular (PrPC), through a process of post-translational conversion without information from nucleic acids. PrPSc seems to act as a template upon which the normal protein PrPC is refolded into a nascent PrPSc molecule. Different types (strains) of infectious prions exist but the species of a particular prion is encoded by the sequence of the PrP gene of the infected animal. In the conversion process, strain-specific properties reside in different tertiary structures (conformations) of PrPSc.

TSE comprise several animal and human clinical entities: Creutzfeldt–Jakob disease (CJD), a dementing encephalopathy with prominent spongiform changes, is probably the most common, but other rarer human TSE are kuru, Gerstmann–Straussler–Scheinker Disease (GSS), FFI and the recently described new variant CJD.

FFI represents the third most frequent genetic prion disease around the world, having been reported worldwide[2] and with similar clinical features also in different genetic backgrounds. Even compared to sporadic CJD, however, which has a worldwide incidence of 1 case per million population per year, FFI remains a rare disease, only about 57 cases in 27 kindreds having been reported in the literature. FFI was identified in 1986[3] because of its peculiar clinical features including prominent abnormalities of the wake-sleep cycle, and has in time proved a model disease for prion infectivity studies and for the neurophysiology of sleep. The principal features of FFI shall be detailed here, with emphasis on the characteristics that make FFI of interest to researchers in the field of sleep physiology. Other reviews of the subject may also be of interest.[2,4]

Clinical features of FFI

FFI is a disease of midlife: symptoms arise at about 51 years of age, though the age of onset ranges from 36 to 62 years. Onset in the twenties is, however possible. Both sexes are equally affected. FFI is uniformly fatal, leading to death within 8–72 months, with a mean of 18 months.[3,5] The disease is progressive, and the course may be short (less than 18 months) or prolonged. Some clinical, metabolic, pathological and genetic differences are associated with these two types of progression[5] [see later]. Most patients complain of initial disturbances of vigilance, like being no longer able to nap, or falling asleep at night, and a feeling of unrefreshing sleep. They may also display some personality changes, like apathy or disinterest. These complaints coincide with the development of visual fatigue with diplopia and sympathetic activation, in the form of unexplained evening pyrexia, subtle hypertension, perspiration, sweating and tearing, and tachycardia/tachypnea. Males become impotent. These initial symptoms are often overlooked and attributed to stress by patients and physicians alike. Both insomnia and autonomic hyperactivity, thereafter, progressively worsen; patients are deprived of refreshing nocturnal sleep and look somnolent during the daytime. Left to themselves, patients may lapse into peculiar episodes of dream enactment, whereby they display complex jerk-like movements mimicking the content of dreams that they are able to recall upon 'awakening', either spontaneously or upon external prodding. These 'oneiric stupor' episodes may occur with open or closed eyes, and initially last only a few seconds. With progression of the disease, recall of the mental content becomes difficult or impossible, and patients become more and more confused, alternating between wakefulness and oneiric confusional states. Middle stages of the disease see the onset of motor impairment

(see later), but some patients may die after only a few months course of isolated autonomic and sleep disturbances, without major motor disability. These patients with a short disease course retain quite normal intellectual abilities when examined during wake. Progressive motor impairment otherwise consists of spontaneous and evoked myoclonus, pyramidal signs, dysmetria and dysequilibrium, dysarthria and dysphagia, and sphincter loss. In later stages of the disease patients become bedridden and speech-less and die in a state of akinetic mutism and emaciation. Patients with a course lasting more than 18 months display relatively fewer sleep and autonomic disturbances, evident, however, upon laboratory investigations, and earlier and more marked, sometimes initial motor impairment. They may show occasional convulsive seizures.

Neuropsychological tests in the original FFI families showed progressive disturbances of attention and vigilance, of working memory and temporal ordering of events. Frontal lobe functions were especially impaired but intelligence was generally preserved at least until vigilance levels allowed meaningful testing. On account of their prominent alterations in alertness and vigilance, it was argued that FFI should not be defined as a dementing, but rather a progressive confusional illness.[6]

Upon routine imaging of the brain, no characteristic features except for variable degrees of cerebral and cerebellar atrophy were detected. Diffusion-weighted or spectroscopy MRI were, however, not done. Brain imaging with [18F]-FDG PET disclosed prominent thalamic hypometabolism sometimes though not always associated with widespread hypometabolism of the basal ganglia, the cortex, especially the fronto-temporal cingular areas, and the cerebellum. The characteristic PET 'signature' of FFI since found in all patients was profound bilateral thalamic with less pronounced cingular hypometabolism (Fig. 1).[7] A degree of hypometabolism was found in some patients also in the baso-lateral frontal and middle and inferior temporal cortices, and the caudate nuclei. Hypometabolism was always more widespread in long disease duration. Comparison with the neuropathological findings showed that the metabolic changes were more widespread than the pathological

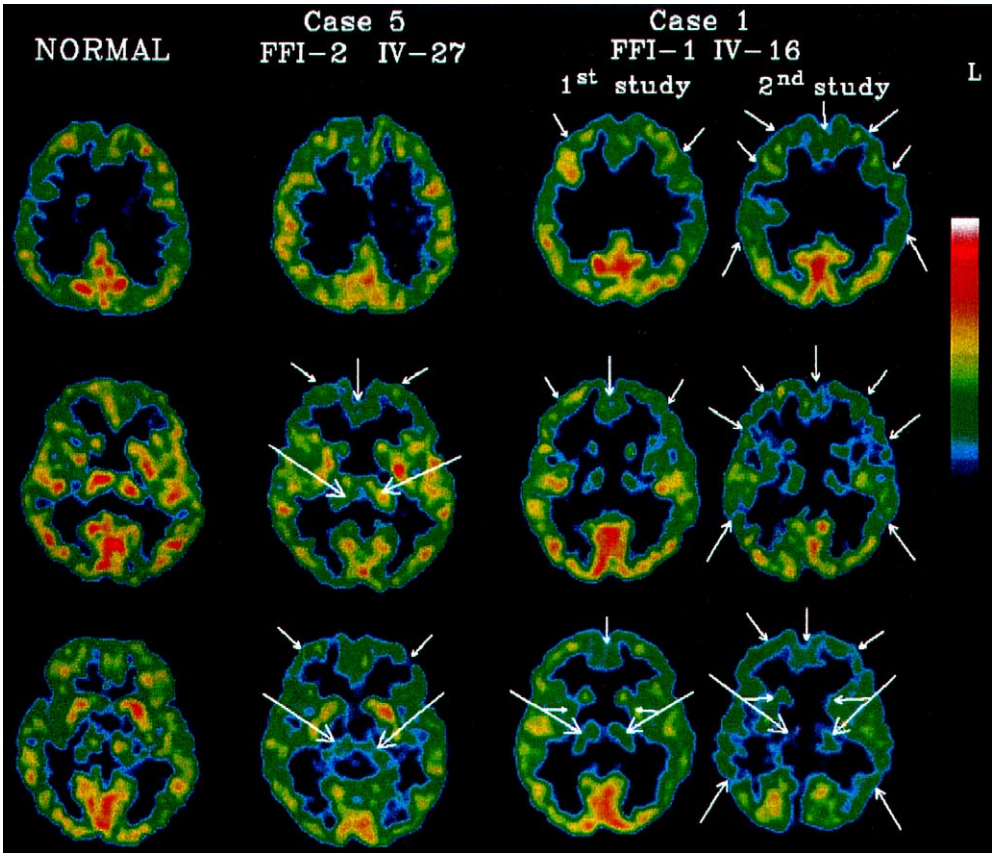

Figure 1 [18F]-FDG PET in a normal subject and FFI patients with a short (case 5) or long-lasting (case 1) disease course, showing hypometabolism in both thalami in case 5, associated with cortical frontal, cingular and temporo-parietal hypometabolism (arrows) more prominent in case 1. With kind permission from Cortelli et al. (1997).

alterations, and were correlated with the amount of abnormal PrPSc deposited in the different brain areas.[7] Functional imaging of the serotonergic system in two FFI patients disclosed reduced availability of the serotonin transporter in thalamic–hypothalamic regions, findings that, given the role of serotonin in sleep physiology, were judged to correlate with the sleep-wake abnormalities.[8] Conventional cerebrospinal fluid (CSF) analysis was generally normal in FFI. The 14-3-3 protein, a good marker of prion diseases, especially sporadic CJD with classical phenotype was found increased in only half of the cases in FFI. High concentrations of the serotonin metabolite 5-OH-indoleacetic acid in the CSF of three FFI patients suggested increased serotonergic activity in FFI.[9] Along with the functional SPECT studies[8] and neuropathological evidence,[10] they implicate the serotonergic system in FFI and more generally in the TSE. CSF hypocretin-1 levels were normal in FFI.[11]

Characteristic sleep disturbances in FFI

Serial EEG and polysomnographic features in the original FFI families were reported by Lugaresi et al.,[3] Tinuper et al.[12] and Sforza et al.[13] In the first reported patient, EEG tracings alternated between two states, one of diffuse alpha activity with normal antigravitary tone and normal responses to presented auditory bursts, corresponding to a behavioural state of wakefulness; the other of desynchronized EEG activity with numerous REMs, tachycardia and irregular breathing, brief or rudimentary atonia on antigravitary muscles and frequent irregular jerks interspersed with more complex motor activity of the limbs, and unresponsiveness to presented auditory bursts (Fig. 2). The latter state corresponded to behavioural sleep but with the patient displaying myoclonic jerks and quasi-purposeful limb gestures. Upon awakening, the patient would report of dreaming. These two states, wakefulness and 'oneiric stupor', alternated during the 24-hours; no sleep figures (K-complexes or sleep spindles) or delta sleep could be observed throughout. Administration of thiopental 250 mg, and diazepam 50 mg could not evoke EEG fast rhythms or features of slow-wave sleep (SWS) (Fig. 3).[12] Later disease stages showed slowed and diminished amplitude monomorphic EEG background activity, unreactive to stimuli, up to flat tracings before death.[12] These findings were replicated in six new FFI cases monitored by longitudinal polysomnographic evaluations every 3–4 months. Somewhat different pictures were detected between short and long duration disease patients. Short evolution patients had severely reduced total sleep time (TST) and wakefulness alternating with brief episodes of abnormal REM sleep, recurring in clusters. While wakefulness was

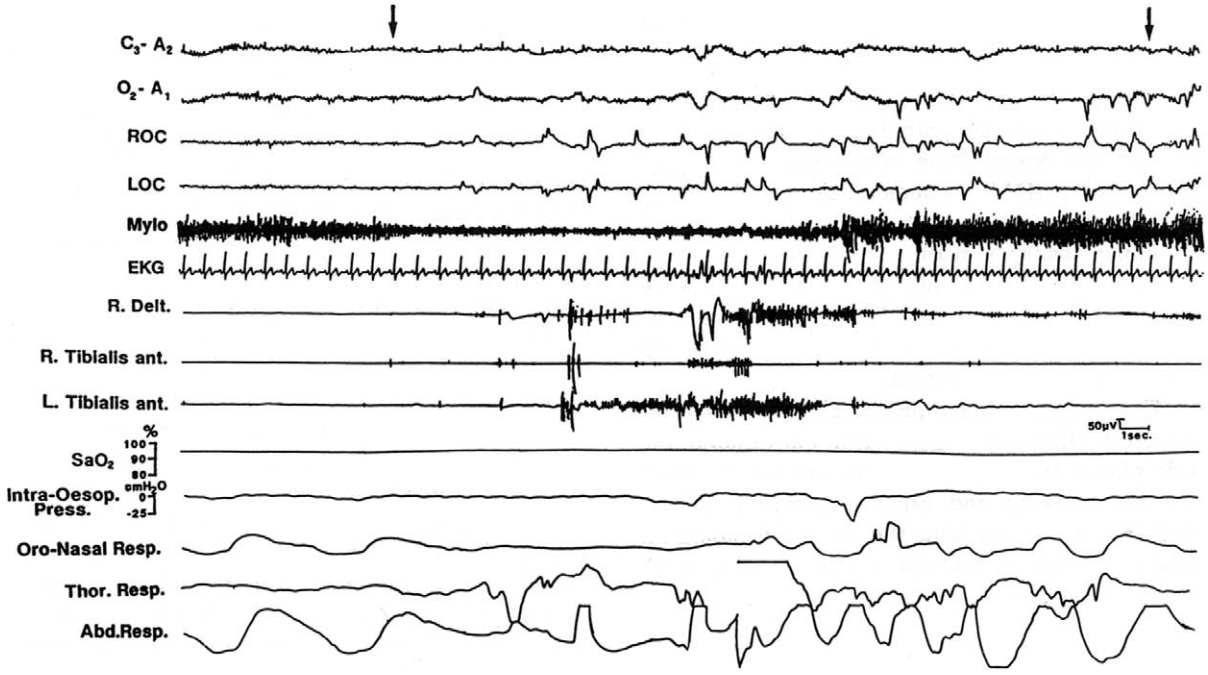

Figure 2 Polygraphic recordings of an enacted dream episode (between arrows) in a FFI patient, showing EEG fast activity of REM sleep, REMs (ROC and LOC) with irregular chin muscle atonia (mylo) and jerks in the limb muscles. From Tinuper et al. (1989) with kind permission from Elsevier.

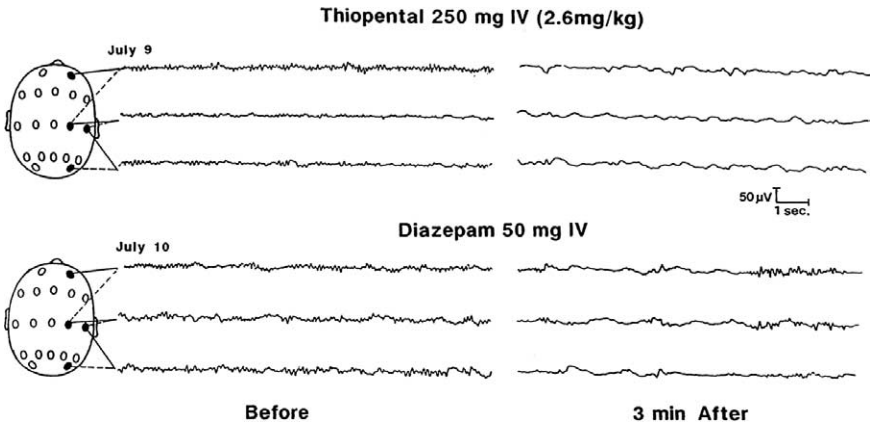

Figure 3 EEG recordings in a FFI patient before and after 250 mg thiopental and 50 mg diazepam intravenous administration. No fast EEG rhythms or SWS could be evoked. From Tinuper et al. (1989) with kind permission from Elsevier.

characterized by EEG alpha activity with intermittent theta trains, abnormal sleep was associated with low amplitude desynchronized EEG, REMs and infrequent and short-lived antigravitary muscle atonia (Fig. 4). SWS (Fig. 5) and sleep figures were conspicuously absent throughout the 24 h.[13] Long evolution patients had prolonged sleep latency, reduced TST and fragmented sleep, with only residual sleep spindles and K-complexes. TST progressively shortened and sleep figures disappeared with disease progression. SWS and REM sleep episodes could abnormally appear directly from wakefulness, the transition marked by a sudden decrease of body temperature and blood pressure and by a sharp build-up of delta activity, or by REM sleep without atonia. Deep and REM sleep percentages progressively decreased to nil in two out of three long evolution patients.[13] Besides loss of SWS sleep and disrupted cyclic organization, the picture common to all patients was judged to be the early and progressive disappearance of sleep spindles (Fig. 6) and K-complexes, impossible to generate even by pharmacologic means. Notably, quasi-periodic sharp wave activity typical of CJD was never recorded in short evolution patients, but in two cases with long evolution just before death.

These abnormal sleep features were associated with autonomic sympathergic activation, with increased body core temperature ($T°$), systemic blood pressure (BP) and heart rate (HR) at rest and enhanced by postural changes, Valsalva and isometric handgrip tests. Plasma noradrenaline levels

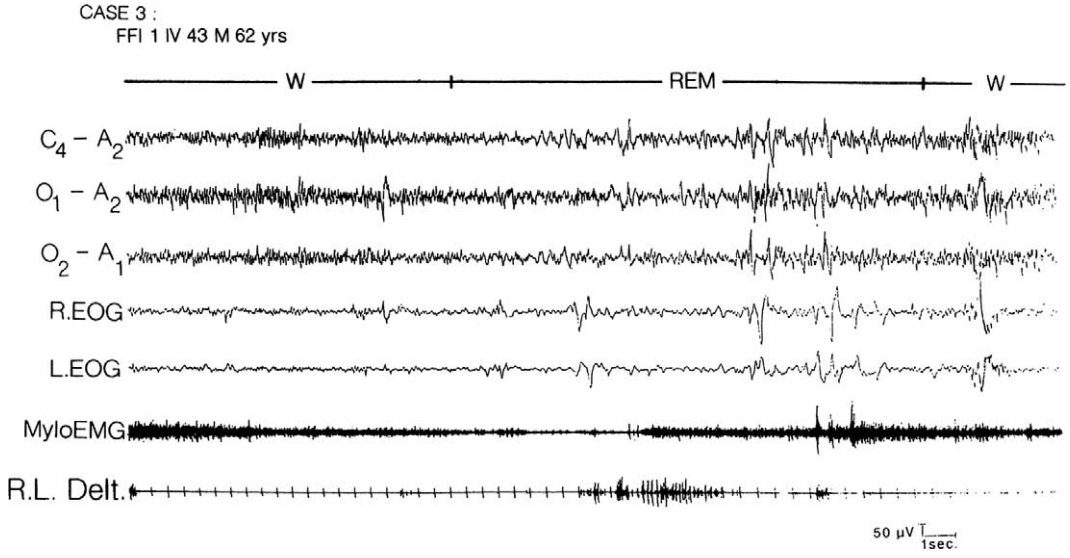

Figure 4 Abnormal REM periods with dream enactement in a FFI patient. Low amplitude desynchronized EEG was associated with REMs (R. EOG and L. EOG) and only rudimentary chin muscle atonia (MyloEMG). From Sforza et al. (1994) with kind permission from Elsevier.

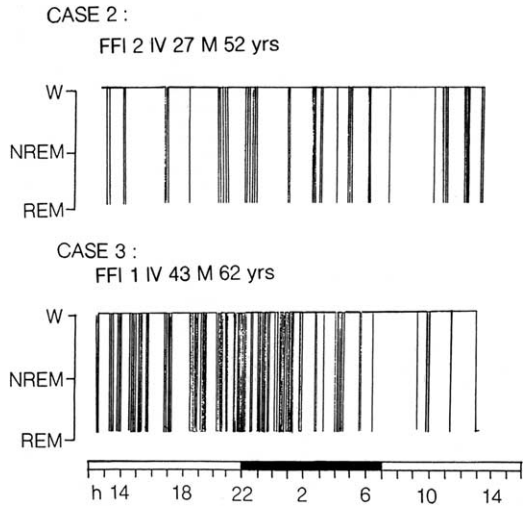

Figure 5 Sleep histograms of 24-hour polysomnographic recordings in 2 FFI patients with a short duration disease course, showing loss of SWS. From Sforza et al. (1994) with kind permission from Elsevier.

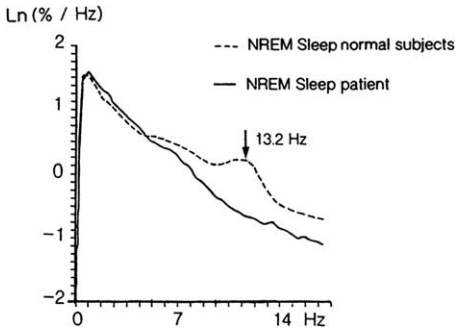

Figure 6 Spectral analysis of EEG traces during stage 2 NREM sleep showing lack of the peak at 13.2 Hz corresponding to sleep spindles in an FFI patient (continuous line) versus a normal subject (dotted line).

were increased at rest and further upon tilt test. Parasympathetic functions were preserved. BP, T° and HR, however, still fell whenever sleep was recorded, thus displaying a normal state-dependent behaviour.

Circadian recordings documented that the dominant 24-hour rhythm of BP and HR was still evident but BP did not fall during the night (Fig. 7).[14] Neuroendocrine studies, while documenting normal responses of the hypothalamic-pituitary axis and target glands to suppression and stimulus tests, showed elevations of serum cortisol in the presence of normal levels of ACTH, and constantly elevated levels of catecholamines over the 24 h (Fig. 8). Consistently with the deep sleep abnormalities, GH showed no nocturnal secretory peaks, while the circadian rhythm of prolactin persisted. Circadian fluctuations, however, tended to disappear with disease progression. Likewise, the nocturnal rise in melatonin was progressively lost (Fig. 8).[15]

Predictably because of the lack of sleep, 52-day actigraphic monitoring in one patient showed up to 80% increased motor activity with a loss of circadian rhythmicity, and indirect calorimetry a staggering 60% metabolic increase in 24-hour energy expenditure.[16]

Neuropathology, genetics and molecular biology of FFI

Neuropathological findings in FFI comprise striking abnormalities in the thalamus, especially affecting the anterior (A) and dorsomedian (DM) thalamic nuclei, and inferior olivary nuclei.[3,17] Thalamic changes were a severe or nearly complete loss of neurons, associated with astrogliosis. Morphometry documented that associative and motor thalamic nuclei lost 90% neurons, and limbic-paralimbic, intralaminar and reticular nuclei lost 60%.[18] Other thalamic nuclei were usually spared, though there could be some involvement of the pulvinar. Spongiform changes were usually absent in the thalamus, though they could be found in the cerebral cortex and underlying white matter, especially in limbic areas. Spongiform changes were generally scant in FFI compared to sporadic CJD, and were observed in cases of long duration. The inferior olives displayed striking loss of neurons and reactive astrogliosis. Some loss of Purkinje and granule cells was found in the cerebellum. Other brain structures were unaffected, though minor and inconstant changes were represented by moderate astrogliosis of the hypothalamus and peri-acqueductal gray matter in the midbrain and basal ganglia.[17,19] Thus neuropathology in FFI can be conceptualized as a preferential thalamo-olivary degeneration. Compared to sporadic CJD, FFI displayed specific vulnerability features, especially involving the thalamic nuclei.[18] Immuno-histochemistry for tryptophan hydroxylase, an enzyme involved in serotonin metabolism, showed increased activity in the median raphe nuclei of eight FFI patients, in the face of conserved numbers of neurons in the median raphe nuclei,[10] an indication that the serotonergic system is abnormal in FFI. The suggestion was offered that these biochemical changes possibly underlie some of the characteristic sleep-wake abnormalities. However, the evidence is for an increased serotonergic activity in FFI, which is at variance with the role of serotonin as a sleep, especially slow-wave sleep inducing molecule; it cannot, therefore, be excluded that these represent changes

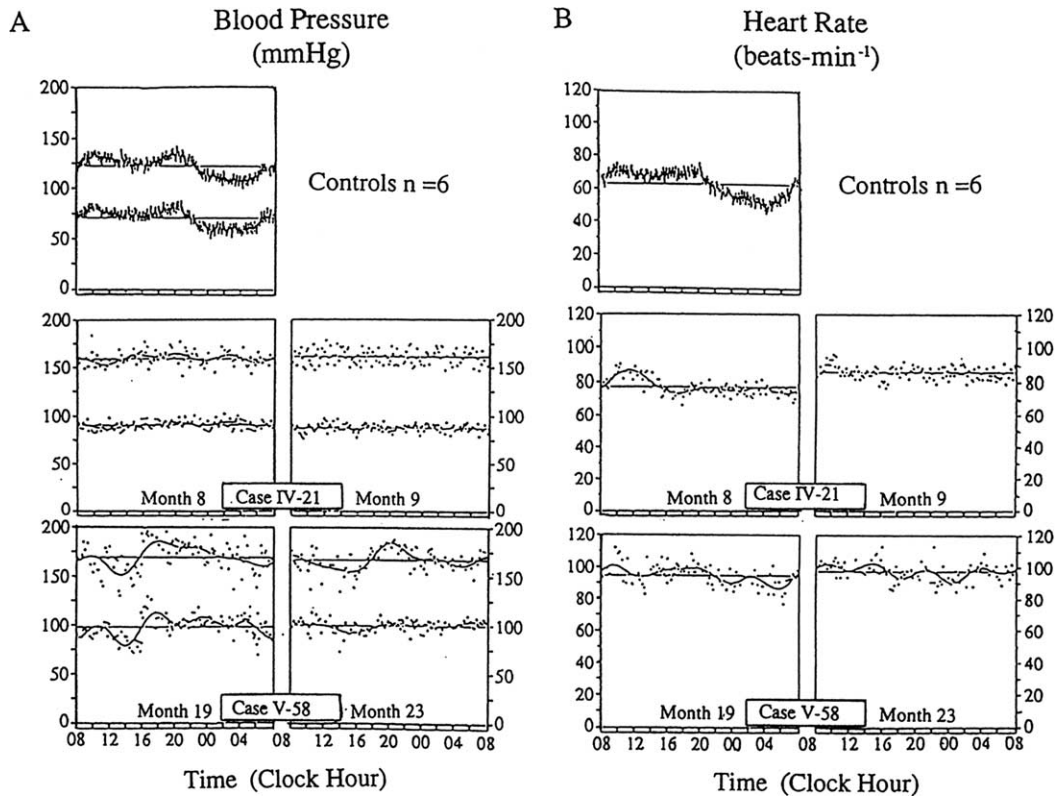

Figure 7 Autonomic circadian rhythms in two FFI patients, (case IV-21 with a short and case V-58 with a long-evolution course) showing the progressive loss of circadian fluctuations especially the nocturnal decrease (see upper inset with findings from 6 controls) in BP (A) and HR (B). From Cortelli et al. (1999) with kind permission.

compensatory and secondary to the sleep abnormalities, namely loss of sleep.

FFI is hereditary, transmitted according to a mendelian autosomal dominant pattern. FFI patients harbour a missense GAC to AAC mutation at codon 178 of the *PRNP* prion protein gene located on chromosome 20.[20] The same mutation was, however, also reported in pedigrees with familial CJD (fCJD) displaying clinical features different from FFI, especially a lack of prominent wake-sleep abnormalities and thalamic atrophy. A clinicopathological comparison between families displaying the typical FFI features versus families with a CJD phenotype (178CJD), all carrying the same 178 codon *PRNP* mutation, disclosed that the modifying factor was the presence in FFI of a methionine polymorphism at codon 129 of the mutated allele of the gene, while valine was instead present at codon 129 in the 178CJD families.[19] Thus, while the 178 codon mutation specifies disease, expression of the FFI phenotype is related to the 129 codon coding for methionine. FFI represented the first instance of a genetic disease in which phenotypic expression was demonstrably related to an intragenic polymorphism.[19] The *PRNP* 129 codon in humans specifies either methionine or valine, and about 42–56% of Caucasians are 129 met/val heterozygotes, 34–49% met/met homozygotes and the remainder val/val homozygotes. The 129 codon polymorphism exerts important modifying influences on prion diseases, whether iatrogenic, sporadic or hereditary. In addition to influences exerted by the 129 codon polymorphism on the mutated allele, in FFI modifying effects could also be exerted by the 129 codon polymorphism on the non-mutated allele: comparison of patients with a short versus prolonged disease showed in fact that the former were usually met/met homozygotes, while the latter met/val heterozygotes.[5] Met/val patients also had fewer sleep abnormalities, more marked motor/sphincter impairment and periodic EEG discharges, and more cortical pathological involvement with spongiosis.[5] Such differentiating features seem, however, not universal.[21]

Deposition of a prion protein insoluble in non-denaturating detergents and resistant to digestion by proteinases (PrPres) represents the hallmark of prion encephalopathies. The normal PrP is found in three different glycosylation patterns: unglycosylated, mono- and di-glycosylated. When PrPres is treated by means of proteinase-K, two major fragments of different sizes are generated

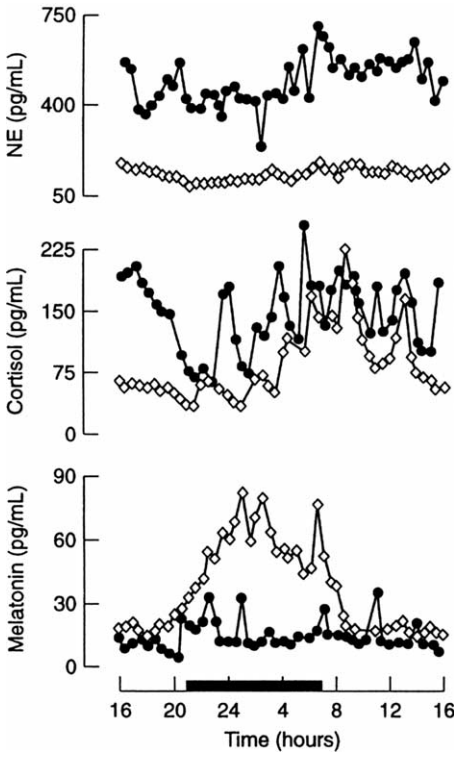

Figure 8 24-hour circadian rhythms in an FFI patient (black circles) versus a normal control (white circles), showing increased levels of noradrenaline (NE) and cortisol and absent melatonin rise during darkness (dark bar). From Cortelli et al. (1999) with kind permission.

presumably because the abnormal protein is cleaved at different sites: a 21 kDa (type 1 PrPSc) and another 19 kDa (type 2 PrPSc) fragment. FFI is characterized by deposition of type 2 PrPSc, while brains of familial 178CJD show deposition of type 1 PrPSc, demonstrating that genetic differences between FFI and 178CJD are also reflected in the abnormal PrPSc proteins.[22] Presumably, the two fragments are cleaved at different sites because of a different conformation of the PrPSc. Glycosylation patterns also differ, with the FFI type of fragment showing under-representation of the unglycosylated form.[22] Moreover, deposition of PrPSc in FFI brains occurs to a much lesser degree than in sporadic CJD,[23] especially in the neo- and limbic cortex of long evolution patients, and amounts correlate with disease duration, whereas deposition in the thalamus is only moderate and unrelated to disease duration. Such a dissociation between the amounts of deposited PrPres and severity of neuronal loss in the thalamus remains problematic, and has been taken to demonstrate a peculiar vulnerability of thalamic structures.[24]

FFI has been transmitted to experimental animals, in particular to transgenic mice engineered to express a chimeric human-mouse PrP gene.[25] Remarkably, upon transmission of the disease from homogenates of FFI brains, the experimental animals displayed deposition of the original type 2 19 kDa PrPSc, while homogenates from familial or sporadic CJD brains caused the deposition of type 1 21 kDa PrPSc.[25] This was the first demonstration that different prion 'strains' exist, and that each PrP isoform acts as a template in inducing a corresponding conformational change for the nascent PrPSc.[26]

Clinical symptoms and pathological changes quite typical of FFI can be found in patients without family history and who do not demonstrate any mutation in *PRNP*. Such patients have been reported under the term Sporadic Fatal Insomnia (SFI).[26,27] All were met/met homozygotes at codon 129 of the *PRNP*, and all had type 2 PrPSc deposited in the brain. Transmission experiments confirmed that the type of PrPSc deposited in the infected animal and the pattern of neuropathological changes, with prominent involvement of the thalamus, were comparable to those obtained with brain homogenates from genetic FFI cases.[26]

These animals were, however, not studied in regard to sleep behaviour. Overall, the genetic and molecular biology studies of FFI have been of great importance for our understanding of the prion diseases. Besides emphasizing the importance of defining the clinical phenotype as accurately as possible, permitting in turn precise correlations with the genotype, they allowed the discovery of the different human strains of prions, shown to be due to different conformations of the abnormal prion protein and able to induce different clinicopathological syndromes. Finally transmission experiments with FFI confirmed the hypothesis of the 'protein' only for prion infectivity, the strain diversity of the prion being enciphered in their conformation independently of any genetic information from the donor.

FFI as a model disease for sleep regulation: (I) the thalamus is essential for the generation of SWS

Clinico-pathological correlations in FFI implicate the thalamus, in particular the A and the DM nuclei, in the characteristic sleep and autonomic disturbances. This is not to negate the role of other brain regions in FFI symptomatology, but pathological changes are overwhelming in the thalamus (and inferior olives), and scant and often inconsistent in other brain regions.

The contention that the thalamus regulates the sleep-wake cycle and more particular the genesis of SWS, put forward by the group in Bologna,[12,13,24] was, however, not new. In pioneering works Hess demonstrated that electrical stimulation of the thalamus, especially the medial thalamus, evoked sleep in the cat.[28] According to Parmeggiani,[29] medial thalamic stimulation induced physiological sleep behaviour in cats. These findings were discounted by authors who negated a functional role of the thalamus in sleep regulation. In particular, Naquet et al.[30] in 6 thalamectomized cats found absent sleep spindles but still present sleep-wake cycles; these animals were, however, followed up for only 25 days. Indeed in later experiments, cats with ablation of the thalamus, so-called 'athalamic' cats, now chronically followed for up to 6 months, showed, besides a permanent absence of sleep spindles, persistent reductions in REM and NREM sleep, which came to represent 2.5 and 11.8%, respectively, of total observation time, compared to 13.8 and 38.2% for intact animals.[31] Remarkably, drowsiness was not affected in these athalamic cats, and sleep spindles were not evoked by thiopental administration; thiopental was, however, able to increase NREM sleep percentages, thus demonstrating the existence of compensatory mechanisms in these animals. Sleep spindles instead persisted, in the face of large reductions in both REM and NREM sleep (residual REM was only 0.65% of total observation time, and NREM sleep $<5\%$) in cats with ablation of the entire neocortex and striatum but intact thalamus, so-called 'diencephalic' cats.[32] These latter animals also displayed absent cyclic distribution of sleep-wake phases, but, again remarkably, drowsiness was not significantly affected. That in Villablanca's experiments decortication abolished SWS to even a greater extent than thalamectomy may represent indeed a relevant finding when discussing the implications of FFI neuropathology, whereby thalamic atrophy is combined with cortical limbic dysfunction. In later research, the focus of interest shifted to the brainstem, basal forebrain and hypothalamus. More recently, however, activity within the DM was shown to be modulated during sleep. Moreover, wakefulness and insomnia (characterized by loss of sleep spindles, significant and gradual reduction in SWS and relative increase in wake time, REM sleep remaining unaffected) follow ibotenic acid lesions of the DM (though not the A) thalamic nucleus.[33] Both the cerebral cortex and thalamus are now thought indispensable for the EEG characteristics of SWS.

Loss of sleep spindles and K-complexes in FFI could be expected since these sleep figures are paced by the thalamus, specifically the nucleus reticularis. It is difficult to ascertain precisely to what extent this nucleus is affected in FFI. However, sleep spindles seem affected by stereotactic lesions in medial thalamic structures in humans and spindle activity in the cat was modified by lesioning the DM nucleus.[34] Anatomical connections between the reticularis and the DM have been traced, in particular the DM receives afferents from the oral pole of the reticular nucleus.[35] Therefore, degeneration of the DM nucleus could be in itself responsible for the loss of spindling activity in FFI. Remarkably, lesioning the reticular nucleus also decreased delta waves and led the animals to sudden death.[36] Loss of spindling activity could thus be all important in FFI, since spindles may be considered a switch between light and deep sleep. Experimental findings are also supported by clinical and functional imaging evidence for an essential role of medial thalamic structures in sleep. Bricolo[37] reported the case of a Parkinsonian patient with persistent insomnia after bilateral stereotactic thalamotomy because of tremor. Patients with paramedian thalamic lesions, in spite of a behavioural presentation with sleep posture, eyes closed and lack of activity, cannot develop NREM and REM sleep states during the daytime. Such a state of 'pseudo-hypersomnia and pre-sleep behaviour' was attributed to 'dearousal' or 'subwakefulness'.[38] Patients with paramedian thalamic strokes (the center of the lesions being the DM) display daytime hypersomnia due to a dearoused state, and increased stage 1 sleep with reduced stage 2 and stages 3–4 NREM sleep and sleep spindles.[39] It was suggested that the paramedian thalamus acts as 'final common pathway' for both maintenance of wakefulness and promotion of NREM sleep.[39] Similar findings of persistent subwakefulness, loss of spindle activity and deep sleep and autonomic activation were reported in paramedian thalamic syndromes associated with calcifications,[40] and peduncular hallucinosis, i.e. visual hallucinations akin to the oneiricism observed in FFI, may occur with thalamic infarction.

Functional neuroimaging has been used to define the brain networks involved in specific behaviours, and is instrumental in elucidating patterns of metabolic activation during sleep. PET demonstrated that synchronized sleep is associated by marked thalamic, among other structures, hypometabolism.[41] Compared with REM, NREM sleep shows significantly lower metabolic rates in the thalamus and, according to Braun et al.[42] profound deactivation occurs during SWS in centrencephalic regions, particularly brainstem, thalamus and

forebrain, paralleled by comparable changes in the activity of limbic and paralimbic areas. These areas (brainstem and thalamus) are also the first to be reactivated upon awakening. Most relevantly, Hofle et al.[43] in a correlation study between spindle and delta activities and brain regional cerebral blood flow (rCBF), found that delta activity covaried negatively with rCBF most markedly in the thalamus and the brainstem reticular formation, cerebellum, anterior cingulate, and orbitofrontal cortex. A significant negative covariation between spindle activity and residual rCBF was also evident in the medial thalamus. These negative covariations were deemed to reflect the disfacilitation and active inhibition of thalamocortical relay neurons in association with delta activity and spindles.

Such physiological patterns of metabolic activity during SWS closely mirror the glucose PET patterns and post-mortem pathological findings in FFI, and provide functional imaging evidence for a fundamental role of the thalamus in SWS generation.

Anatomical data indicate that, more generally, the DM and A thalamic nuclei are interposed in the circuitry linking the limbic areas to the hypothalamus and basal forebrain. The DM nucleus represents an intermediate station in the trans-basal ganglionic circuit of the limbic system in a network interconnecting the ventral striatum and pallidum with the prefrontal cortex. The DM receives inputs both from the hypothalamus[44] and the basal forebrain,[35] with direct GABAergic projections from the basal forebrain and preoptic-anterior hypothalamic regions to the 'intermediate' part of the DM nucleus in the cat.[44] As a way of explaining the clinical features of FFI, it was, therefore, hypothesized that thalamic lesions introduce a diaschisis between limbic regions involved in instinctive behaviour and those cortical-subcortical areas—basal forebrain, hypothalamus, brainstem—that promote sleep, allowing for a functional imbalance to arise in the form of sympathergic activation and loss of SWS.[24] The thalamo-limbic circuits have been indeed conceptualized as areas of neural representations of motivational drives, including the need for sleep.

Finally, the DM forms part of the so-called central autonomic network, which regulates and integrates all autonomic functions and a role for setting the sympathetic tone via hypothalamic neurons has been claimed for the medial (but not the lateral) thalamus. Bicuculline injections in the DM reproduce some autonomic features of FFI, in particular they cause hypertension and tachycardia through removal of GABAergic inhibition.[45] From all this a coherent view emerges of medial thalamo-limbic structures as mechanistically involved in SWS production and autonomic balance.

FFI as a model disease for sleep regulation: (II) SWS loss, autonomic and motor hyperactivity and the concept of 'Agrypnia Excitata'

Loss of sleep associated with hyperactivation of motor and autonomic functions is not encountered in FFI alone. Severe, even complete loss of sleep, polygraphically documented, was reported under the term of 'agrypnia' ('αγρυπνια=to chase sleep away), which is ordinarily used for cases of organic insomnia, e.g. insomnia due to lesions of the CNS. The term was applied to some infectious diseases such as trypanosomiasis, but especially to Morvan fibrillary chorea, a disease characterized by even complete loss of sleep and now recognized as an autoimmune encephalitis especially involving the limbic areas.[46] Morvan fibrillary chorea comprises peripheral neuromyotonia associated with autonomic hyperactivity and central abnormalities in the form of severe insomnia and oneirism. While neuromyotonia is due to antibodies against voltage-gated K+ channels, and though there is evidence for autoimmune activation within the CNS, the exact agent for the agrypnia in Morvan disease is still unknown. Autoimmune agrypnia with purely CNS manifestations has been reported.[47] Loss of SWS is also seen in delirium tremens, the alcohol withdrawal syndrome, which combines lack of NREM especially SWS with oneirism and prominent even fatal autonomic activation (tachycardia and tachypnea, hypertension and perspiration) with extreme motor agitation. Thus Morvan fibrillary chorea and delirium tremens bear intriguing similarities to FFI. Moreover, there is evidence, even though limited, that both conditions affect the functionality of the thalamus. On these grounds, the term 'Agrypnia Excitata' (AE) was put forward[48] to account for the association of SWS loss ("agrypnia") and abnormal REM sleep (oneirism) with autonomic and motor overactivation ("excitata"), and it was proposed that the sleep-wake and autonomic abnormalities characteristic of AE are underscored by dysfunction within the thalamo-limbic circuits. AE may also be categorized as a particular instance within the broader concept of Status Dissociatus, primarily defined by the presence of ambiguous, multiple, or rapid oscillation of state-determining variables. Though the characteristic oneirism and REM sleep

features of AE bear resemblances to the REM sleep behaviour disorders (RBD), RBD remain unassociated with profound loss of SWS and do not display the prominent motor and autonomic activation observed over the 24 h in AE. AE has been proposed as a useful concept in clinical neurology, and initial conceptual evidence can be gathered that AE could also apply to some psychiatric conditions such as mania, again displaying features of agrypnia and motor and autonomic hyperactivity.

FFI as a model disease for sleep regulation: (III) 'light' versus 'deep' sleep and the concept of NREM sleep

One intriguing feature of FFI is that SWS together with the transitional figures of sleep (sleep spindles, K-complexes) that herald delta sleep are remarkably lost, while stage 1 NREM sleep is conserved. Traditionally NREM sleep is viewed as a continuous monochord process starting from stage 1 and progressing to stage 4, a view encapsulated within the current standard of sleep scoring. However, historically the incorporation of stage 1 NREM into sleep proper was contentious from the beginning. Indeed, normal subjects often report being awake during stage 1 NREM[49] and cognitive responses to external stimulations decrease significantly only during stages 3–4 NREM and REM sleep.[50] The findings in FFI suggest that 'light' sleep, until the appearance of sleep spindles, should probably more meaningfully be conjoined with relaxed wakefulness under the concept of sleep onset, and that its underlying mechanisms are different from those generating spindle activity and SWS and probably unrelated to thalamic functioning.[48,51] This view is consistent with the conceptualization of sleep as an instinctive behaviour,[52] whereby its initial appetitive phase was attributed to reticular deactivation[52] or to mechanisms placed within limbic structures.[29] This appetitive phase, that we could now assimilate to drowsiness/stage 1 sleep, is followed by the consummatory phase with its cyclic organization of SWS and REM sleep, whose mechanisms relate to activity within neural structures different from the appetitive phase.[52] That differing properties and neural mechanisms underlie stage 1 versus SWS is supported clinically by some neurophysiological phenomena (slow eye movements, hypnic jerks, propriospinal myoclonus at the wake-sleep transition) peculiar to stage 1 sleep, and by experimental findings, whereby dissociation between rest and true sleep was effected by brain lesioning in animals.[53,54] Phylo-ontogenetic arguments also suggest that while poikilothermic animals present behaviours mimicking drowsiness or pre-sleep behaviour, true SWS and REM sleep evolved only in homeotherms. The neural structures originating these pre-sleep and sleep behaviours are different. Villablanca[54] postulated the existence of an independent dual control for sleep and wakefulness, by the rostral brain ('*brain sleep* and wakefulness') and caudal brainstem structures ('*body sleep* and wakefulness'): the rostral brain originating SWS, the brainstem being involved with body rest without true sleep (i.e. drowsiness). Three different types of sleep: drowsiness/stage 1 NREM, REM sleep and SWS, developing according to a caudo-rostral organization were proposed by Lugaresi et al.[51] The findings in FFI and AE, conditions where an 'appetite' for sleep is preserved but 'true sleep' cannot be consumed, add to the literature emphasizing the independence of stage 1 sleep mechanisms from those originating SWS. Accordingly, it seems inappropriate to conflate stage 1 with SWS stages into a unique NREM sleep process, and the concept of NREM sleep should be reappraised.

FFI as a model disease for sleep regulation: (IV) a role for prions in sleep?

The physiological significance of the normal prion protein is still unsettled, but it was envisaged that the prion protein intervenes in sleep regulation. Some support for the contention that PrP itself regulates sleep may be indeed gathered from the literature on animals infected with prion inoculates which display a wide range of sleep-wake disturbances. Overall, changes in sleep organisation in inoculated animals occurred early, with the beginning of the clinical phase and at the end of the incubation period. Interestingly, minks suffer from a naturally occurring prion encephalopathy that, upon transmission to the Syrian hamster, causes two clinical syndromes, one HYPER characterized by hyperexcitability (HY syndrome) and the other one DROWSY with progressive lethargy and drowsiness (DY). These two contrasting phenotypes relate to two PrPres strains with different kinetics and with strain specific patterns of localization and neuron damage,[55] in a way very reminiscent of the transmission studies of FFI versus fCJD. Monitoring of circadian behaviour in mice inoculated with TSE revealed abnormal locomotor patterns most pronounced during the nocturnal active phase

which were strain specific and were seen well in advance of clinical signs. There is also sporadic evidence that human patients with CJD display sleep disturbances: precocious loss of physiological sleep, replacement of physiological states of sleep by cyclic changes consisting of EEG periodic complexes alternating with theta-delta activity, excessive sleepiness and abnormal sleep architecture have all been reported in sporadic and iatrogenic cases of CJD.[56–59] The hypothesis that *PRNP* is involved in sleep regulation was tested by Tobler et al.[60] who studied sleep characteristics in *PRNP* knockout mice (129/SV). Baseline recordings showed no difference in the amount of vigilance; the null mice, however, exhibited a larger degree of sleep fragmentation with almost double the amount of short waking episodes, and faster increase in slow-wave activity at waking to NREM sleep transitions. Lower 0.25–5.0 Hz frequencies were reduced throughout all vigilance states. During recovery after sleep deprivation, the PrP-null mice displayed a larger and longer lasting increase in 0.75–4.0 Hz activity in NREM sleep. These findings were taken to indicate that PrP promotes sleep continuity[60] and the lower peak frequency within the theta band during REM sleep and waking in the null mice suggested that PrP may be affecting the theta-generating mechanisms in the hippocampus during waking and REM sleep. In a later study, however, Huber et al.[61] argued that since the larger increase in slow wave activity after sleep deprivation in PrP null mice was restricted to the occipital derivations and appeared after the waking-NREM sleep transitions, PrP seemed unlikely to be involved in the mechanisms enabling the transition to sleep; rather, differences could be due to the need for recovery in this particular brain region.[61] In a polysomnographic study of the originally reported FFI family comprising 17 healthy relatives, eight carriers of the 178 FFI mutation versus nine non-carriers, no statistically significant difference was found in the absolute and relative power for the delta, theta, alpha and sigma EEG bands or, for that matter, in other macrostructural sleep characteristics.[62] These findings indicated that sleep changes in FFI become evident only upon clinical onset of the disease, in agreement with PET studies showing normal values of thalamic metabolism even a short time before onset of symptoms. In a second study of spectral EEG characteristics comparing 129 met/val versus 129 met/met carriers, spindle frequency band power and the ratio between delta and spindle activity were found to correlate with 129 *PRNP* codon polymorphism, irrespective of the 178 codon mutation.[63] If replicated in larger samples, these findings would implicate the 129 polymorphism in the regulation of spindling and delta activity. No involvement of the 129 codon polymorphism was, however, detected in a study of the clinical and polysomnographic features of a sample of insomniac patients by Pedrazzoli et al.[64] Alongside similar controversial evidence of the involvement of the 129 codon polymorphism in other neurodegenerative diseases, cognitive performance and cognitive deterioration in the elderly, the role of the *PRNP* gene in sleep regulation must still be considered unsubstantiated and in need of further experimental confirmation.

Controversial features and conclusions

Apart from our ignorance of the pathological mechanisms underlying FFI, which reflects our more general ignorance of the pathogenesis in prion diseases, some features and findings in FFI remain controversial, or at least in need of confirmation. Controversy exists on whether sleep-wake abnormalities are universal features or specific to FFI or whether they may also be found in other TSE.[2] Insomnia and EEG changes observed in prion encephalopathies other than FFI seem, however, to depend upon the existence of thalamic lesions,[65,66] and sleep abnormalities were absent in GSS, a prion disease characterized by different *PRNP* mutations and no thalamic pathology,[67] thus in the end confirming the FFI model. Also controversial remains whether part of the phenotypic variability in FFI actually depends on the 129 *PRNP* codon polymorphism on the non-mutated allele.[21] Misunderstandings may be caused by the apparent discrepancy between patients' subjective reports of inability to have a fully refreshing sleep (insomnia) and objective observations of a drowsy behaviour simulating hypersomnia (but which cannot progress to a veritable SWS). It must be emphasized that the term 'insomnia' in FFI relates to the polygraphically proven inability to develop SWS. Other inconsistencies in the literature are clearly due to the lack of detailed clinical observations and especially the absence of polygraphic monitoring in some patients. Conceivably, however, part of the phenotypic variability of FFI relates to biological factors still to be discovered. Questionable remains also whether part of the FFI symptomatology is due to the consequences of sleep deprivation, especially since experimental sleep deprivation has been found to reproduce some of the metabolic characteristics of FFI. In the

end, while the pathogenesis of FFI and other prion diseases remains elusive, the most pressing need is represented by treatment, which in such a severe disease is simply unavailable or ineffective.

Practice points

(1) FFI is a hereditary prion disease due to a 178 codon mutation in the *PRNP* gene cosegregating with a Met polymorphism at codon 129 of the mutated allele. SFI has similar clinical and pathological features in the absence of *PRNP* mutations.
(2) FFI or SFI should be diagnostic hypotheses in patients with progressive loss of SWS and autonomic activation.
(3) Similar clinical features are observed in cases of limbic encephalitis and delirium tremens (the concept of 'Agrypnia Excitata').

Research agenda

FFI sets important tasks for future sleep research:

(1) The neural connectivity and biochemical mechanisms underlying the workings of the thalamo-limbic areas during SWS and their possible involvement in homeostasis of SWS and sleep deprivation, need elucidation at the cellular and molecular levels.
(2) A reconceptualization of NREM sleep has major consequences in the clinical setting (e.g. sleep scoring, classification of sleep disorders, etc.).
(3) Genetic and other factors modifying FFI phenotypic expression (among others the 129 codon polymorphism on the non-mutated *PRNP* allele) need further study.
(4) The biological function of the PrP protein, and its eventual role in sleep physiology, are still unclear.
(5) Finally and most relevantly, therapeutic approaches should be explored for these rare but terrible diseases.

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