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Hippocampal bursts caused by changes in NMDA receptor-dependent excitation in a mouse model of variant CJD

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ABSTRACT

Prion diseases are heterogeneous in clinical presentation, suggesting that different prion diseases have distinct pathophysiological changes. To understand the pathophysiology specific to variant Creutzfeldt–Jakob Disease (vCJD), *in vitro* electrophysiological studies were performed in a mouse model in which human-derived vCJD prions were transmitted to transgenic mice expressing human instead of murine prion protein. Paired-pulse stimulation of the Schaffer collaterals evoked hypersynchronous bursting in the hippocampus of vCJD-inoculated mice; comparable bursts were never observed in control or *Prnp* knockout mice, or in mice inoculated with a strain of prion associated with classical CJD. Furthermore, NMDA receptor-mediated excitation was increased in vCJD-inoculated mice. Using pharmacological experiments and computer simulations, we demonstrate that the increase in NMDA receptor-mediated excitation is necessary and sufficient to explain the distinctive bursting pattern in vCJD. These pathophysiological changes appear to result from a prion strain-specific gain-of-function and may explain some of the distinguishing clinical features of vCJD.

Introduction

Prion protein is expressed ubiquitously throughout the nervous system. The role of its normal isoform (PrP^C) remains unclear, although it has been implicated in pre-synaptic function (Herms et al., 1999), oxidative stress protection (Brown et al., 1997b), cation binding (Brown et al., 1997a; Jackson et al., 2001), calcium buffering (Powell et al., 2008) and signal transduction (Lopes et al., 2005). The pathogenic isoform (PrP^{Sc}) plays a key role in several neurodegenerative diseases including fatal familial insomnia and multiple forms of Creutzfeldt–Jakob Disease (CJD) in humans, as well as scrapie and bovine spongiform encephalopathy in other animals (Prusiner, 1998).

Despite their common connection with PrP^{Sc}, prion diseases have different clinical features. For example, variant CJD (vCJD) is unique in several respects: it affects younger people than classical CJD (cCJD), its duration is more than double that of cCJD (medians 14 and 5 months, respectively), it lacks EEG abnormalities characteristic of cCJD, and it is characterized by a long period of psychiatric abnormalities before neurological symptoms typical of cCJD and scrapie develop (Spencer et al., 2002; Zerr and Poser, 2002; Wieser et al., 2006). Differences in clinical features are paralleled by subtle molecular variations in PrP. Indeed, it is now well established that different strains of prion cause clinically distinct diseases (Monari et al., 1994; Collinge et al., 1996; Telling et al., 1996; Parchi et al., 1996; Safar et al., 1998; Hill et al., 2003; Tanaka et al., 2004; Wadsworth and Collinge, 2007).

The diversity of prion strains and their associated diseases have important practical implications for animal models of human disease: in order to replicate a specific human disease, the appropriate strain of prion must be transmitted to an appropriate animal. In that regard, extrapolating from a mouse model for scrapie to infer pathophysiological mechanisms involved in one or another human prion disease is problematic. However, because of the risk of infection when working with human-derived prions, all previous electrophysiological studies have investigated animal forms of prion disease, e.g. scrapie in rodents. Some studies revealed hypersynchrony and abnormal membrane currents (Bassant et al., 1987; Jefferys et al., 1994; Johnston et al., 1997, 1998a; Barrow et al., 1999) while others indicated loss of synaptic function (Johnston et al., 1998b; Belichenko et al., 2000; Chiti et al., 2006; Mallucci et al., 2007). However, the connection between these specific pathophysiological changes and a specific human prion disease remains unclear.

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To study the pathophysiology associated specifically with vCJD, human-derived vCJD prions were transmitted *in vivo* to transgenic mice that express human instead of murine PrP. Electrophysiological changes were studied *in vitro* using a hippocampal slice preparation from infected animals. Computer simulations were used to reproduce the experimentally observed pattern of bursting and to test our experimentally derived explanation of bursting. Through this combined approach, we have identified a distinctive pattern of bursting in vCJD and have explained its synaptic basis. Comparisons with *Prnp* knockout and cCJD-inoculated mice demonstrate that the pathophysiological changes are unique to vCJD.

Materials and methods

Animal models

Data were obtained from transgenic mice expressing normal human PrP instead of the murine PrP. These animals (FVB152×sv129 Bl/6, Tg (HuPrP$^{+/+}$, *Prnp*$^{0/0}$)-152) have been thoroughly described (Whittington et al., 1995) and used previously to study human prion diseases (Hill et al., 1997). Mice received 30 µl intracerebroventricular inoculations of brain homogenate from (1) a patient who died of vCJD (I348, Type 4, *Prnp* 129 MM), (2) a patient who died of classical CJD (I1215, Type 2, *Prnp* 129 MM), (3) a control post-mortem brain or (4) phosphate buffered saline. In total, we examined 31 mice at 148–752 days after inoculation with vCJD homogenate, 29 mice at 196–316 days after inoculation with classical CJD homogenate, and 41 mice at 37–716 days after control inoculation (30 control brain extract and 11 saline; we found no qualitative or statistically significant difference between those two sets of controls and data were therefore pooled). We also examined 17 *Prnp* knockout mice (Sv129×C57Bl6×FVB background), which did not receive any inoculation.

The health hazard posed by human-derived prions required that the inoculated mice were housed in cages contained in double-HEPA filtered isolators and were manipulated with modified forceps, and all experiments were performed in containment facilities to level 3 with derogations for infective prions. Mice were monitored at least twice a week for neurological signs which included: inactivity and behavioural submission; pilar, penile, tail, and ear erection; ataxia; hunched posture and abnormal gait; weight loss; sleep disorders. Severe signs included breathing irregularities and periods of generalized tremor or immobility. Two or more symptoms displayed consistently over 48 h were considered clinically diagnostic of prion disease. Adequate measures were taken to minimize pain and discomfort and mice were used for electrophysiology before this criterion was reached if they displayed signs of distress. Experiments were performed under the UK Animals (Scientific Procedures) Act of 1986, with the approval of the Institutional Ethics Committees, and in accordance to international standards on animal welfare.

Electrophysiological recordings

Mice were killed by cervical dislocation under halothane anaesthesia at various times after inoculation (445±27 days after inoculation for vCJD mice and 232±6 days after inoculation for cCJD mice, which corresponds to 90% and 114% of full incubation times for vCJD and cCJD, respectively). Dissection and preparation of brain slices using a modified Vibroslice ROMA (Campden Instruments, Sileby, UK) were performed in a double-HEPA filtered isolator to contain the biohazard. 400 µm-thick slices were cut parasagitally and submerged in a storage chamber at room temperature. For recording, slices were moved to an interface chamber at 32±1 °C perfused (2 ml/min) with artificial cerebrospinal fluid composed of (in mM): NaCl, 125; KCl, 3; $NaHCO_3$, 26; NaH_2PO_4, 1.25; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 10; pH 7.4.

Intracellular responses in CA1 pyramidal cells were recorded using sharp glass electrodes (40–80 MΩ) filled with 2 M potassium acetate. Signals were amplified using an Axoclamp-2B (Axon Instruments, Burlingham, CA, USA) DC amplifier. Resting membrane potential of CA1 neurons was adjusted to −65 mV. Field potentials in various CA1 areas were recorded using 2–5 MΩ glass pipettes filled with 2 M NaCl. Field potentials were filtered (2 kHz) and amplified using Neurolog AC-coupled NL 104 preamplifiers (Digitimer Ltd, Welwyn, UK). Both intracellular signals and field potentials were filtered (2 kHz) and digitized (5 kHz) using a CED 1401 computer interface (Cambridge Electronic Design, Cambridge, UK). Stimuli (0.1 ms duration) were delivered with a twisted 50 µm Ni/Cr wire bipolar electrode. Unless otherwise specified, the stimulating electrode was placed in the Schaffer collateral zone 500 µm from the recording site. To isolate CA1 from possible epileptogenic activity in CA3, in some cases a cut was made between the two regions. Drugs were purchased from Tocris (Bristol, UK). Analysis was performed using Signal and Spike2 software (CED, Cambridge, UK). Data are reported as mean±SEM. Statistical tests are indicated in the text.

Computer simulations

Simulations were performed with NEURON simulation software (Carnevale and Hines, 2006). Our approach was to simulate as simple a neural network as might plausibly support hypersynchronous activity. Model pyramidal cells were identical and were based on a reconstructed CA1 pyramidal neuron (Pyapali et al., 1998) with 160 compartments. A passive leak conductance was distributed uniformly throughout the neuron to produce an input resistance of 100 MΩ and resting membrane potential of −65 mV. Model basket cells were based on a single somatic compartment with a passive leak conductance adjusted to produce an input resistance of 350 MΩ and resting membrane potential of −65 mV. Fast sodium and delayed rectifier potassium conductances based on Traub and Miles (1991) were inserted into the soma of each cell type at a density of 300 and 60 mS/cm^2 and into the pyramidal cell dendrites at one tenth those densities.

One AMPA and one NMDA synapse was inserted in the apical dendrite, another three AMPA synapses and three NMDA synapses were inserted in the basal dendrites, and one $GABA_A$ synapse was inserted in the soma of each pyramidal cell. A single AMPA synapse was inserted in each basket cell. Synaptic conductances were modeled as a rapid exponential rise in conductance combined with a slower exponential decay in conductance described by τ_{rise} and τ_{decay}, respectively. Synaptic current is therefore written as

$$I_{syn}(t) = w\,[1-\exp(-t/\tau_{rise})]\exp(-t/\tau_{decay})(V_m - E_{rev})$$

where $t=0$ at the onset of a synaptic event, w is synaptic weight, V_m is membrane potential, and E_{rev} is reversal potential. For AMPA synapses, $\tau_{rise}=0.2$ ms, $\tau_{decay}=2$ ms, and $E_{rev}=0$ mV; for $GABA_A$ synapses, $\tau_{rise}=0.8$ ms, $\tau_{decay}=8$ ms, and $E_{rev}=-70$ mV as in previous modeling (Prescott and De Koninck, 2003). NMDA synaptic kinetics were extrapolated from Hestrin et al. (1990) and were $\tau_{rise}=6$ ms, $\tau_{decay}=60$ ms, and $E_{rev}=0$ mV; voltage-sensitivity was modeled after Jahr and Stevens (1990a,b) such that w is scaled by $\alpha(V)$ where

$$\alpha(V) = 1/\left(1 + e^{-0.062V}\left[Mg^{2+}\right]_o/3.57\right)$$

and $[Mg^{2+}]_o=1$ mM. Simulations with greater numbers of heterogeneously weighted synapses per cell were performed but results were qualitatively unchanged from the model with fewer synapses; results from the simpler model are reported throughout. Synaptic weights were adjusted to give reasonably sized synaptic responses, and were varied between cells so that each cell responded slightly differently to stimulation. Once synaptic weights were set, variation in stimulus intensity and pathological changes were simulated by uniformly scaling weights of subsets of synapses (see below).