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

Stéphanie Ratté a,1, Steven A. Prescott b,1, John Collinge c, John G.R. Jefferys a,∗

a Department of Neuroscience (Neurophysiology), Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
b Computational Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA
c MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurosurgery and Neurology, Queen Square, London, WC1N 3BG, UK

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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.

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Introduction

Prion protein is expressed ubiquitously throughout the nervous system. The role of its normal isoform (PrPC) 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 (PrPSc) plays a key role in several neurodegenerative diseases including familial Creutzfeldt–Jakob Disease (CJD) in humans, as well as scrapie and bovine spongiform encephalopathy in other animals (Prusiner, 1998).

Despite their common connection with PrPSc, 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.
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-/-)-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 (1348, Type 4, Prnp 129 MM), (2) a patient who died of classical CJD (11215, 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 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 parasagittally 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; NaHCO3, 26; NaH2PO4, 1.25; CaCl2, 2; MgCl2, 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/cm2 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

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

where τ = 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, τ rise = 0.2 ms, τ decay = 2 ms, and E rev = 0 mV; for GABA A synapses, τ rise = 0.8 ms, τ 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 τ rise = 6 ms, τ decay = 60 ms, and E rev = 0 mV; voltage-sensitivity was modeled after Jahr and Stevens (1990a,b) such that w is scaled by α(V) where

$$ \alpha(V) = \frac{1}{\left(1 + e^{-0.0625V [Mg^{2+}]_{o}}\right)} / 3.57 $$

and [Mg2+]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).
Paired-pulse stimulation of the Schaffer collaterals was simulated as synaptic excitation (AMPA and NMDA) in the apical dendrite of the pyramidal neurons and synaptic excitation of a basket cell, which in turn caused feedforward inhibition (GABA_A) in the soma of the pyramidal neurons. Synaptic excitation resulting from Schaffer collateral stimulation occurred at a 1 ms delay while all other synaptic activity (feedforward inhibition, feedback inhibition, and recurrent excitation) was delayed 5 ms from the time of action potential generation in the soma of the pre-synaptic neuron. Each pyramidal neuron connected to each other pyramidal cell through the three AMPA and three NMDA synapses in the basal dendrites, thereby providing excitatory feedback. Each pyramidal cell also connected to two of the three basket cells, which in turn connected to the GABA_A synapse on two of the three pyramidal cells, thereby providing feedback inhibition. Synaptic connectivity is illustrated in Fig. 6A.

Paired-pulse stimulation was tested at three intervals (10, 100, and 1000 ms) and at three intensities (moderate intensity and 50% above and below moderate intensity, i.e. ±0.5x). Stimulus intensity was varied by adjusting the strength of the synaptic connections associated with Schaffer collateral input. Pathological changes in receptor density/function were simulated by scaling the strengths of all NMDA synapses and/or GABA synapses onto pyramidal neurons (no distinction was made between NMDA synapses involved in Schaffer collateral input vs. recurrent excitation, or between GABA synapses involved in feedforward inhibition vs. feedback inhibition). Effects of changing the strength of AMPA receptors was also tested.

Results

Variant CJD is associated with a unique pattern of bursting in CA1 hippocampus

Since this was the first electrophysiological study of a vCJD mouse model, we utilized an exploratory protocol that reveals information about both excitatory and inhibitory synaptic transmission at the network level by recording local field potentials in hippocampal area CA1 following single and paired-pulse stimulation of the Schaffer collaterals (Fig. 1A). Of 28 vCJD-inoculated mice (at 233, 280, 403, 539, 598, 720, and 752 days post-inoculation), paired-pulse stimulation triggered prolonged hypersynchronous bursting after the second stimulus (Fig. 1B). The same stimulus protocol never elicited bursting in control mice (n = 36), in Prnp knockout mice (n = 17), or in CJD-inoculated mice (n = 29). The correlation between bursting and vCJD inoculation, summarized in the inset on Fig. 1B, is thus highly significant (Fisher’s exact test; p = 0.005 for vCJD vs. control or CJD; p < 0.05 for vCJD vs. Prnp knockout). Since bursting is uniquely associated with vCJD, the remainder of this paper focuses on characterizing and explaining those bursts. A detailed investigation of pathophysiology associated with CJD is beyond the scope of this study but, suffice it to say, the pathophysiological changes in vCJD differ categorically from those in CJD, and do not result from a simple loss of PrP function.

The bursts observed in vCJD-inoculated mice (henceforth “vCJD mice”) were distinctive and different from epileptiform bursts such as those caused by disinhibition. Bursts were never elicited by a single Schaffer collateral stimulus and they arose within CA1, which was verified by separating CA1 and CA3 with a cut, during the experiment in some slices but before the start of the experiments in most slices. In contrast, bursts associated with focal epilepsy can be elicited by a single stimulus (see Discussion) and typically arise in CA3 (Miles et al., 1984). Furthermore, bursts in vCJD were elicited only with interpulse intervals between 50 and 200 ms, and only by moderate amplitude stimuli. These unique requirements for bursting limit the possible mechanisms that may underlie it (see below).

Intrinsic neuronal properties appeared unchanged in vCJD mice. Average resting membrane potential was unaltered (∼56.6 ± 1.9 mV in vCJD vs. ∼56.3 ± 1.6 mV in control) and input resistance was not significantly different (37.4 ± 2.9 MΩ in vCJD vs. 43.5 ± 2.9 MΩ in control; t-test, 18 neurons for vCJD and 21 for control). Neither the slow nor the medium afterhyperpolarizing potentials (AHPs) following a train of 12 action potentials were different: medium AHP (measured at 40 ms) was 3.2 ± 0.5 mV in vCJD vs. 4.3 ± 0.7 mV in control and slow AHP (measured at 200 ms) was 2.2 ± 0.5 mV in vCJD vs. 3.1 ± 0.6 mV in control (t-tests; 8 cells in vCJD and 10 in control).

Bursts lasted as long as 1.7 s (normal responses lasted 3–6 ms) and were all-or-none, i.e. paired-pulse stimulation with the appropriate parameters (see above) elicited full sized bursts whereas bursts were completely absent for stimulation outside the appropriate parameter range. The all-or-none nature of bursting indicates a qualitative change in network behaviour which, based on the lack of significant changes in intrinsic neuronal properties, most likely resulted from
changes in synaptic transmission. Field potential recordings from the stratum oriens showed a large negativity during the burst (Fig. 1C) indicating strong excitatory synaptic input onto the basal dendrites, consistent with strong recurrent excitation between pyramidal neurons. Simultaneous intracellular recordings from pyramidal neurons confirmed that bursting was associated with rapid spiking driven by excessively strong excitatory synaptic input; example in Fig. 1C shows depolarization block and a dramatic increase in total membrane conductance resulting from that synaptic drive (note the lack of voltage responses to current pulses marked "*").

**Paired-pulse facilitation and NMDA receptor-mediated excitation are increased in vCJD**

Our next step was to quantify changes in synaptic transmission that give rise to bursting in a subset of vCJD slices. Since bursting required paired-pulse stimulation, we first quantified paired-pulse facilitation as the ratio of the second to first population spike (Fig. 2A). Facilitation was significantly larger in vCJD (p<0.005, ANOVA; Fig. 2B). Subdividing vCJD slices based on whether they were taken from a mouse in which at least one slice exhibited bursts or from a mouse in which no slices exhibited bursts revealed that paired-pulse facilitation was significantly greater in the former at the 100 ms interpulse interval (p<0.05, t-test) (Fig. 2C). An increase in paired-pulse facilitation at intermediate interpulse intervals (i.e. the same 50–200 ms intervals eliciting bursts) thus seems to be correlated with bursting in vCJD slices.

To determine the basis for differences in paired-pulse facilitation, we investigated the response elicited by a single stimulus (Fig. 3A). The population spike amplitude was actually smaller in vCJD than in control slices (p<0.001, ANOVA; Fig. 3B, top panel), although the stimulus–response curves were roughly the same after being normalized by the maximal response (Fig. 3B, bottom panel). A reduction in population spike amplitude cannot account for the enhanced paired-pulse facilitation and bursting in vCJD. However, we also noticed that the extracellularly recorded synaptic response was significantly longer in vCJD (Fig. 3A) with a decay tau of 7.6±0.3 ms compared with 6.0±0.5 ms in control (p<0.05, t-test; Fig. 3C). Prolonged synaptic excitation might explain the enhanced paired-pulse facilitation and bursting in vCJD (see below).

There are two likely explanations for the prolongation of net synaptic excitation: either synaptic inhibition is decreased or slow synaptic excitation is increased. To test whether inhibition was reduced, we measured the inhibitory postsynaptic potential (IPSP) in CA1 pyramidal cells after stimulation of the Schaffer collaterals (Fig. 4A); we stimulated each cell with various stimulus amplitudes, and report single IPSP amplitudes. The IPSP measured 30 ms after stimulation (i.e. the average latency to the peak of the GABA₆ receptor-mediated voltage response (Davies et al., 1990)) was significantly smaller in vCJD (5.3±0.8 mV, n=4) than in control (8.2±0.5 mV, n=5) (p<0.005, ANOVA). The IPSP measured at its peak was similarly smaller in vCJD (5.5±0.8 mV) than in control (8.8±0.5 mV; p<0.001, ANOVA). However, since slow synaptic excitation could affect amplitude of the compound IPSP, we applied NBQX (20 µM) and D-AP5 (25 µM) to block ionotropic excitatory potentials and measured the pure IPSP elicited by direct stimulation of inhibitory interneurons in the stratum oriens or stratum radiatum (Fig. 4B). Under these conditions, the IPSP in vCJD (15.3±0.3 mV for SO, n=8, and 16.3±0.5 mV for SR, n=10) was not significantly different from control (15.1±0.2 mV for SO, n=8, and 15.5±0.6 mV for SR, n=7; ANOVAs). These data suggest, therefore, that inhibitory interneurons may be less strongly activated by Schaffer collateral stimulation but that the inhibitory synapses themselves continue to function normally in vCJD; in other words, synaptic inhibition is not directly impaired in vCJD although it...
abolished by NMDA receptor blockade. Indeed, D-AP5 reduced whether the enhanced paired-pulse facilitation in vCJD was no longer statistically different from control (1.32±0.12) after D-AP5 (paired t-test). Nonetheless, the paired-pulse ratio at 100 ms was no longer significantly different between vCJD (1.22±0.11) and control (1.32±0.12) after D-AP5 (t-test; compare with significant difference in Fig. 2C).

Increased NMDA receptor-mediated excitation is necessary and sufficient to explain bursting

The next obvious step was to test whether NMDA receptor blockade would prevent bursting. Indeed it did, in all four slices tested (Fig. 5E). Thus, increased NMDA receptor-mediated excitation completely accounts for the difference in local field potential kinetics between vCJD and control, explains the difference in paired-pulse facilitation, and is necessary for bursting in vCJD. But is increased NMDA receptor-mediated excitation sufficient to explain bursting and, in particular, can it explain the unique pattern of bursting associated with vCJD (i.e. elicited only after the second stimulus, only for intermediate interpulse intervals, and only for moderate amplitude stimulation)?

To address the issue of sufficiency, we built a computer model to explore the effects of enhanced NMDA receptor-mediated excitation...
on network activity (Fig. 6A). With synaptic weights set to produce the response pattern observed in control mice, bursting was not elicited for stimulation at short, intermediate, or long intervals (10, 100, and 1000 ms, respectively) or for stimulation with weak, moderate, and strong stimulus intensities (top, middle, and bottom rows). (C) Moderate strengthening (25–35%) of NMDA synapses caused bursting in response to moderate stimulation at 100 ms interval (grey box), which is consistent with the experimentally observed pattern of bursting in vCJD mice. Greater strengthening caused bursting in response to strong stimulation at 100 ms interval, inconsistent with experimental observations. (D) With the NMDA synaptic weights set to control values, moderate weakening of GABA synapses caused bursting in response to stimulation at 10 ms interval, which is inconsistent with the vCJD pattern of bursting. Further weakening led to bursts in response to the first of the paired stimuli. (E) Strengthening NMDA synapses while weakening GABA synapses also led to the experimentally observed pattern of bursting (grey box). Therefore, strengthening of NMDA synapses is necessary and sufficient to explain bursting in response to the second of two moderate intensity stimuli presented at intermediate intervals, although concurrent weakening of GABA synapses may reduce the minimum strengthening of NMDA synapses required to cause bursting. (F) Strengthening AMPA synapses did not cause bursting. Only AMPA synapses on pyramidal neurons were strengthened in order not to activate inhibitory neurons more strongly. (G) Reconstructed CA1 pyramidal neuron showing sites in the apical dendrite, soma, and basal dendrite for which membrane potential and synaptic conductances are shown. Traces on left show responses for synaptic weights set to control values; traces on right show responses with NMDA strength increased by 30%. In both cases, stimulation of the Schaffer collaterals caused depolarization in the apical dendrites that spread to the soma and elicited a spike before the onset of feedforward inhibition in the soma. Other pyramidal neurons within the network behaved the same way (data not shown), causing strong recurrent excitation amongst the pyramidal neurons, which is evident from the resultant depolarization in the basal dendrites. However, because of the pre-existing inhibition within the soma, even strong depolarization in the basal dendrites did not elicit spiking in the soma, except under one condition, when NMDA receptor-mediated excitation in the basal dendrites outlasted GABA_α receptor-mediated inhibition in the soma.

Fig. 6. Increased NMDA receptor-mediated excitation is sufficient to explain bursting in vCJD. (A) Computer model was generated as described in Materials and methods. Wiring diagram of network is shown here. Triangles represent pyramidal cells and large circles represent basket cells. (B) With synaptic weights set to control values, bursting did not occur for any set of stimulus parameters. For parts B–F, traces show responses from a single neuron within the network. Time of paired-pulse stimulation is indicated by arrowheads below each trace. Testing was repeated for paired-pulse intervals of 10, 100, and 1000 ms (left, middle, and right columns in each panel) and, for the 100 ms interval, at weak, moderate, and strong stimulus intensities (top, middle, and bottom rows). (C) Moderate strengthening (25–35%) of NMDA synapses caused bursting in response to moderate stimulation at 100 ms interval (grey box), which is consistent with the experimentally observed pattern of bursting in vCJD mice. Greater strengthening caused bursting in response to strong stimulation at 100 ms interval, inconsistent with experimental observations. (D) With the NMDA synaptic weights set to control values, moderate weakening of GABA synapses caused bursting in response to stimulation at 10 ms interval, which is inconsistent with the vCJD pattern of bursting. Further weakening led to bursts in response to the first of the paired stimuli. (E) Strengthening NMDA synapses while weakening GABA synapses also led to the experimentally observed pattern of bursting (grey box). Therefore, strengthening of NMDA synapses is necessary and sufficient to explain bursting in response to the second of two moderate intensity stimuli presented at intermediate intervals, although concurrent weakening of GABA synapses may reduce the minimum strengthening of NMDA synapses required to cause bursting. (F) Strengthening AMPA synapses did not cause bursting. Only AMPA synapses on pyramidal neurons were strengthened in order not to activate inhibitory neurons more strongly. (G) Reconstructed CA1 pyramidal neuron showing sites in the apical dendrite, soma, and basal dendrite for which membrane potential and synaptic conductances are shown. Traces on left show responses for synaptic weights set to control values; traces on right show responses with NMDA strength increased by 30%. In both cases, stimulation of the Schaffer collaterals caused depolarization in the apical dendrites that spread to the soma and elicited a spike before the onset of feedforward inhibition in the soma. Other pyramidal neurons within the network behaved the same way (data not shown), causing strong recurrent excitation amongst the pyramidal neurons, which is evident from the resultant depolarization in the basal dendrites. However, because of the pre-existing inhibition within the soma, even strong depolarization in the basal dendrites did not elicit spiking in the soma, except under one condition, when NMDA receptor-mediated excitation in the basal dendrites outlasted GABA_α receptor-mediated inhibition in the soma.
those observed in vCJD and eventually led to bursting evoked by a single stimulus (Fig. 6D), which is consistent with past work on the effects of disinhibition (Schwartzkroin and Prince, 1977; MacDonald and Barker, 1978; Schwartzkroin and Prince, 1980). Since changes in glutamatergic and GABAergic transmission are not mutually exclusive and may interact (Tancredi et al., 1990; Traub et al., 1994), we tested the effects of changing both: strengthening NMDA synapses while weakening GABA<sub>A</sub> synapses produced the appropriate pattern of bursting (Fig. 6E). With weak GABA<sub>A</sub> synapses, the minimum NMDA strengthening necessary to produce bursting was less than with GABA<sub>A</sub> synapses at full strength (compare with Fig. 6C). Strengthening AMPA synapses did not cause bursting (Fig. 6F).

According to these results, an increase in NMDA receptor-mediated excitation is sufficient to explain bursting. Our simulations cannot rule out contributions from other mechanisms but likely possibilities (e.g. reduced GABA<sub>A</sub> receptor-mediated inhibition) cannot, on their own, account for the distinctive pattern of bursting associated with vCJD. Furthermore, our experimental data clearly show an increase in NMDA receptor-mediated excitation (Fig. 5) while arguing against increased AMPA receptor-mediated excitation (Fig. 3B), decreased GABA<sub>A</sub> receptor-mediated inhibition, or increased neuronal excitability (see above).

Our next step was to use the model to explain how NMDA receptor-mediated excitation causes this distinctive pattern of paired-pulse-elicited bursting. To this end, Fig. 6G shows the membrane potential in the apical dendrite, soma, and basal dendrite of a pyramidal neuron within the network, together with the synaptic conductances at each of those sites. For both control (left panel) and enhanced NMDA (right panel), synaptic excitation elicited by Schaffer collateral stimulation causes depolarization of the apical dendrite that propagates to the soma and elicits a spike before the onset of feedforward inhibition. Despite sustained depolarization in the apical dendrite (especially in the presence of strong NMDA synapses), somatic inhibition prevents the generation of additional spikes. The single spike nonetheless causes recurrent excitation of the other pyramidal neurons, as well as feedback inhibition via basket cells. Recurrent excitation causes depolarization in the basal dendrites, but whether this depolarization elicits somatic spiking depends on the balance between excitation and inhibition; notably, the GABA<sub>A</sub> conductance caused by feedforward inhibition to the soma is still strong when recurrent excitation arrives in the basal dendrites. The balance between excitation and inhibition shifts in favor of excitation after the second stimulus because of the larger contribution from NMDA receptors, which are unblocked by depolarization produced by the first stimulus and have slow kinetics. If excitation is sufficient to cause dendritic depolarization that outlasts somatic inhibition, additional spikes will be generated in the soma and will propagate recurrent excitation which, if not controlled by feedback inhibition, results in bursting.

Sensitivity of bursting to stimulus amplitude and interpulse interval are also explained by the model. Bursting does not occur in response to strong stimulation because the dendritic depolarization caused by excitation tends to saturate whereas the shunting caused by inhibition sums linearly, thereby shifting the balance of excitation and inhibition in favor of inhibition. The same is true for moderate intensity stimulation at short intervals, where temporal summation leads to dendritic saturation. On the other hand, weak stimuli may be insufficient to initiate recurrent excitation, let alone propagate it. For stimulation at long intervals, depolarization caused by the first input has waned when the second input occurs, so that NMDA receptors are no longer unblocked at the start of the second pulse. It is, therefore, moderate amplitude stimulation at intermediate intervals that is most likely to elicit bursting after the second pulse.

**Discussion**

This is the first study to investigate how electrophysiological properties change in a model of a specific human prion disease. Our data revealed a novel form of hypersynchronous bursting in the CA1 region of hippocampus of “humanized” mice infected with vCJD. Our data also revealed an increase in NMDA receptor-mediated excitation in vCJD mice. Pharmacological experiments demonstrated that this increase was necessary for bursting, while computer simulations confirmed that it was sufficient to explain the distinctive pattern of bursting observed in vCJD mice.

Previous studies have implicated NMDA receptors in prion diseases, but results have been inconsistent. For instance, Diez et al. (2001) demonstrated increased NMDA expression levels (inferred from MK-801 binding) in the hippocampus of scrapie-infected mice in the early stages of disease, although expression was decreased in later stages. The latter observation is consistent with decreased NMDA receptor immunoreactivity observed post-mortem in CJD patients (Ferrer and Puig, 2003). But no changes in NMDA receptor-mediated responses were seen in scrapie-infected hamsters (Johnston et al., 1998a), although studies in cultured neurons found that neuronal death induced by toxic PrP fragments or PrP<sup>Sc</sup> was largely mediated by NMDA receptor activity (Muller et al., 1993; Sassoon et al., 2004). Clinically, the drug flupirtine, whose effects include blocking the NMDA receptor, has been shown to have beneficial effects on the cognitive function of CJD patients (Otto et al., 2004), which is consistent with its cytoprotective effects in cultured neurons (Perovic et al., 1995). However, equivalent data specific for vCJD are not available.

Discrepancies regarding the role of NMDA receptors likely reflect variations in the pathophysiology associated with different prion diseases and models. For example, Johnston et al. (1997) showed that paired-pulse facilitation was normal in scrapie-infected mice, whereas we observed a significant increase in paired-pulse facilitation in our vCJD-infected mice. Moreover, Johnston et al. reported depolarized resting membrane potential and increased input resistance in neurons from scrapie-infected mice, whereas we observed no significant difference in neurons from vCJD-infected mice. The two animal models are, quite simply, modeling different diseases. Prion diseases are clinically diverse and are associated with different prion strains (see Introduction). From this, one may well expect that pathophysiological mechanisms vary from one prion disease to another. Our results showing the complete absence of bursts in mice infected with a strain of prion associated with classical CJD support that conclusion. Furthermore, the NMDA receptor-dependent bursting described here does not result from a simple loss of function, since Prnp knockout mice did not exhibit bursting after paired-pulse stimulation.

Broadly speaking, NMDA receptor function is implicated in many neurodegenerative diseases primarily through its role in excitotoxicity (Beal, 1992; Choi, 1992; Lipton and Rosenberg, 1994). Indeed, histopathological features of prion diseases suggest that excitotoxic mechanisms are involved in the neurodegeneration (Scallet and Ye, 1997). NMDA receptors have also long been implicated in the generation of epileptiform activity (Herron et al., 1985; Dingledine et al., 1986; Mody et al., 1987). Hypersynchronous bursting has been described in some prion diseases (Brown et al., 1986; Bassant et al., 1987; Jefferys et al., 1994), although its features were distinct from the paired-pulse-elicited bursts described here.

The precise mechanism responsible for the increase in NMDA receptor-mediated excitation in vCJD remains unclear. Modulation of NMDA receptors by glycine (Forsythe et al., 1988), divalent cations (Westbrook and Mayer, 1987; Tremblay and Shepherd, 1996; Kim et al., 2002) and oxidation–reduction reactions (Gozlan and Ben Ari, 1995; Sanchez et al., 2000; Lipton et al., 2002) might be affected by the inflammatory response (Bettoumi et al., 1996) or by PrP dysfunction in vCJD (Brown et al., 1999; Wong et al., 2001) with consequences for channel function. Alternatively, changes in the levels of growth hormones related to the inflammatory response (Walsh et al., 2001; Cunningham et al., 2002) can increase the density of NMDA receptors; for instance, insulin, whose levels were recently found to be elevated.
in scrapie-infected cells (Nielsen et al., 2004), potentiates NMDA-mediated activity in hippocampus by increasing the number of NMDA receptors delivered to the membrane (Liu et al., 1995; Skeberdis et al., 2001). Sasson et al. (2004) have also reported a change in NMDA receptor subunit expression in cultured neurons after exposure to toxic PrP fragments.

The pattern of bursting in vCJD is unique insofar as it occurs only after the second of two stimuli separated by a 50–200 ms interpulse interval. To the best of our knowledge, such a pattern has not been previously described. Our computer modeling suggests that, beyond a simplistic balance of excitation and inhibition, the temporal properties of synaptic excitation and inhibition are critical for burst generation. This is highlighted by the observation that strengthening AMPA synapses did not cause bursting. Whereas AMPA receptor-mediated excitation is brief, NMDA receptor-mediated input is prolonged and, if strong enough, can cause depolarization that outlasts GABA receptor-mediated inhibition. Notably, the NMDA conductance is larger on the second pulse (and is therefore more liable to produce bursting after the second pulse) because NMDA receptor channels have become unblocked by depolarization produced by the first stimulus. The minimal activation of NMDA receptor channels after the first pulse might explain why we only see a change in the decay time constant after the first pulse but no increase in amplitude in our field potentials (Fig. 3). Furthermore, an increase in NMDA receptor-mediated excitatory response amplitude could be offset in our field recordings by a decrease in the AMPA-receptor mediated excitatory response (because of degeneration and fewer neurons being active). Although the amplitude did not noticeably increase in response to the first pulse, the relative amplitude of the excitatory potential did increase in response to the second pulse in vCJD (i.e. paired-pulse ratio was increased in vCJD mice (Fig. 2)). This is consistent with our hypothesis that the second pulse is more likely to activate NMDA-mediated receptors. Strengthening of NMDA synapses may allow the second stimulus to activate recurrent excitatory pathways (Thomson and Radora, 1991). From the theoretical literature on attractor networks, slow excitation is crucial for stably maintaining persistent activity (Wang, 1999; Compte, 2006) which, for our purposes, equates with sustaining the burst. Thus, the bursting mechanism we describe is consistent with the general principles associated with generation of hypersynchronous activity (Traub and Jefferys, 1994) but is unique in its temporal properties.

The enhanced NMDA receptor-mediated excitation that we have observed after inoculation with a cCJD-associated prion strain or in knockout mice, suggesting that different prion diseases have caused by inoculation with a vCJD-associated prion strain were not observed after inoculation with a cCJD-associated prion strain or in Ptrp knockout mice, suggesting that different prion diseases have distinct underlying pathophysiology. Such understanding is crucial for mechanistically linking molecular pathologies with their clinical manifestation. These data implicate NMDA-dependent bursting specifically in the pathogenesis of variant CJD.

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References


