RBM3 mediates structural plasticity and protective effects of cooling in neurodegeneration

Diego Peretti1, Amandine Bastide1, Helois Radford1, Nicholas Verity1, Colin Molloy1, Maria Guerra Martin1, Julie A. Moreno1, Joern R. Steinert1, Tim Smith1, David Dinsdale1, Anne E. Willis1 & Giovanna R. Mallucci1,2

In the healthy adult brain synapses are continuously remodelled through a process of elimination and formation known as structural plasticity1. Reduction in synapse number is a consistent early feature of neurodegenerative diseases2,3, suggesting deficient compensatory mechanisms. Although much is known about toxic processes leading to synaptic dysfunction and loss in these disorders2,3, how synaptic regeneration is affected is unknown. In hibernating mammals, cooling induces loss of synaptic contacts, which are reformed on rewarming, a form of structural plasticity4,5. We have found that similar changes occur in artificially cooled laboratory rodents. Cooling and hibernation also induce a number of cold-shock proteins in the brain, including the RNA binding protein, RBM3 (ref. 6). The relationship of such proteins to structural plasticity is unknown. Here we show that synapse regeneration is impaired in mouse models of neurodegenerative disease, in association with the failure to induce RBM3. In both prion-infected and 5XFAD (Alzheimer-type) mice7, neurodegenerative disease, in association with the failure to induce RBM3. In both prion-infected and 5XFAD (Alzheimer-type) mice7, how synaptic regeneration is affected is unknown. In hibernating mammals, cooling induces loss of synaptic contacts, which are reformed on rewarming, a form of structural plasticity4,5. We have found that similar changes occur in artificially cooled laboratory rodents. Cooling and hibernation also induce a number of cold-shock proteins in the brain, including the RNA binding protein, RBM3 (ref. 6). The relationship of such proteins to structural plasticity is unknown. Here we show that synapse regeneration is impaired in mouse models of neurodegenerative disease, in association with the failure to induce RBM3. In both prion-infected and 5XFAD (Alzheimer-type) mice7, neurodegenerative disease, in association with the failure to induce RBM3. Enhanced expression of RBM3 in the hippocampus prevented this deficit and restored the capacity for synapse reassembly after cooling. RBM3 overexpression, achieved either by boosting endogenous levels through hyperthermia before the loss of the RBM3 response or by lentiviral delivery, resulted in sustained synaptic protection in 5XFAD mice and throughout the course of prion disease, preventing behavioural deficits and neuronal loss and significantly prolonging survival. In contrast, knockdown of RBM3 exacerbated synapse loss in both models and accelerated disease and prevented the neuroprotective effects of cooling. Thus, deficient synapse regeneration, mediated at least in part by failure of the RBM3 stress response, contributes to synapse loss throughout the course of neurodegenerative disease. The data support enhancing cold-shock pathways as potential protective therapies in neurodegenerative disorders.

We used the phenomenon of physiological structural plasticity seen in hibernating mammals to determine the capacity for synapse regeneration in mouse models of neurodegenerative disease. When they enter torpor, the neurons of hibernators undergo morphological changes including changes in spine morphology8,9 and/or changes in connectivity8,9. These are rapidly reversed on regaining normal body temperature4,5,8–10. We first established that the phenomenon of synapse dismantling and reassembly (structural plasticity) on artificial cooling and rewarming occurs in laboratory mice (Fig. 1a and Extended Data Fig. 1a). We then explored the capacity for structural plasticity after cooling in two mouse models of neurodegenerative disease: prion disease and the 5XFAD model of Alzheimer’s disease10. We used tg37+/− mice11 infected with Rocky Mountain Laboratory (RML) prions used in our previous studies12–14. These mice show substantial synapse loss from 7 weeks post-inoculation.

Figure 1 | The capacity for synaptic regeneration is lost early in neurodegenerative disease. a, Synapse numbers decline on cooling and recover on rewarming in wild-type mice, counted in both 3D and 2D. Representative electron micrographs (pseudo-coloured for ease of synapse identification: yellow, presynaptic; green, postsynaptic compartments) and bar charts showing quantification are shown for each experiment (n = 4 animals at 18 °C and n = 2 at 37 °C; 192 images from 2 mice per condition for 3D analyses; 93 images from 3 animals per condition, for 2D analyses). A typical tripartite synapse is shown at higher magnification. b, c, The same response is seen in prion-diseased mice (b) at 4 and 5 w.p.i. but this fails at 6 w.p.i (arrow), and in 5XFAD (c) mice, where it fails at 3 months (arrow). ***P < 0.0001, **P < 0.01; NS, not significant. Student’s t-test; two tailed. All data in bar charts are mean ± s.e.m. Scale bar, 1 μm. Source Data for all figures can be found in the Supplementary Tables.

1Medical Research Council Toxicology Unit, Hodgkin Building, University of Leicester, Lancaster Road, Leicester LE1 9HN, UK. 2Department of Clinical Neurosciences, Clifford Allbutt Building, Cambridge Biomedical Campus, University of Cambridge, Cambridge CB2 0AH, UK.
All mice were cooled to 16–18 °C for 45 min, similar to core temperatures reached in small hibernators (deep hypothermia) using the biomolecule 5′-adenosine monophosphate (5′-AMP)\(^{15}\), after which they were allowed to slowly rewarm. Animals were euthanized at each stage of the cooling–rewarming process and synapses were counted in the CA1 region of hippocampus. Both synapse density and total synapse number significantly declined on cooling, but recovered on rewarming in wild-type mice, as measured using both three dimensional (3D)\(^{14}\) and two dimensional (2D) analyses\(^{2}\) (Fig. 1a). Neither brain volume nor synapse size changed on cooling and rewarming, excluding the possibility that changes in synapse density reflected changes in these parameters (Extended Data Fig. 1a). Thus, wild-type mice showed synaptic structural plasticity with reduction in synapse number on cooling and recovery on rewarming (Fig. 1a). This capacity for plasticity was also seen in both prion-infected and 5XFAD mice very early in the course of disease, at 4 and 5 w.p.i., and at 2 months of age, respectively (Fig. 1b, c). However, this capacity was lost by 6 w.p.i. in prion-diseased mice (Fig. 1b and Extended Data Fig. 1b) and at 3 months in 5XFAD mice (Fig. 1c and Extended Data Fig. 1c). Notably, impaired structural plasticity shortly preceded established decline in synapse number seen in prion-infected tg37\(^{+}/+\) mice at 7 w.p.i. (ref. 14), and in the 5XFAD mice from 4 months of age (see schematic, Extended Data Fig. 1d). The lost ability to reassemble synapses was not due to loss of synaptic proteins at this stage (Extended Data Fig. 2) nor to increased levels of disease-specific misfolded proteins.
prion protein (PrPSc) in prion-infected mice, or of amyloid-β oligomers in 5XFAD mice induced by the cooling–rewarming process (Extended Data Fig. 3).

In hibernation and hypothermia, global protein synthesis and cell metabolism are downregulated, but low temperature also induces a small subset of proteins known as cold-shock proteins that escape translational repression. Amongst these, RNA-binding motif protein 3 (RBM3) and cold-inducible RNA binding protein (CIRP, also known as CIIBP) are cold-shock proteins expressed at high levels in brain. We found strong induction of RBM3 by cooling in brains of wild-type mice and in mice with prion disease at 4 w.p.i. and 5XFAD mice at 2 months (Fig. 2). CIRP was not upregulated (Extended Data Fig. 4). However, both prion and 5XFAD mice lost the capacity to upregulate RBM3 after cooling at 6 w.p.i. (Fig. 2b) and at 3 months of age (Fig. 2c), respectively, in parallel with the lost ability to reassemble synapses after cooling at these time points (Fig. 1b, c). Therefore, we asked if induction of RBM3 expression drives synaptic recovery.

Therapeutic hypothermia is a powerful neuroprotectant in brain injury acting through multiple mechanisms, including enhanced gene expression driving regenerative processes enhancing synapse formation (see ref. 17 for a review). RBM3 has been implicated in protection against cell death in various in vitro models of cooling and neuroprotection, albeit in conditions of mild hypothermia (32°C). It is known to increase local protein synthesis at dendrites and global protein synthesis through ribosomal subunit binding and/or microRNA biogenesis. The neuroprotective effects of hypothermia on neurodegenerative disease are unknown, however. Given that the capacity for structural plasticity correlated with induction of RBM3, we asked if raising endogenous RBM3 levels through early therapeutic cooling would restore failed synaptic plasticity. In wild-type mice, a single episode of cooling to 16–18°C raised RBM3 levels in brain for up to 3 days (see Fig. 2a and Extended Data Fig. 4c), suggesting the response is sustained for some time after the cold stress. Animals were cooled twice: at 3 w.p.i. and again at 4 w.p.i., resulting in a sustained several-fold increase in RBM3 expression up to 6 weeks later, declining to baseline levels at 12 w.p.i., at the terminal stage of disease (Fig. 3a and Extended Data Fig. 5). Control mice were infected with prions but were not cooled. Early cooling and associated increased RBM3 expression protected against synapse loss in prion disease at 7, 8 and 9 w.p.i. (Fig. 3b), restored synaptic transmission (Fig. 3c) and prevented behavioural deficits, maintaining burrowing behaviours and novel object recognition memory (Fig. 3d and Extended Data Fig. 6a). There was also marked neuronal protection in the hippocampus (Fig. 3e, compare subpanels ii and iii), even in mice succumbing to prion infection, which is ultimately overwhelming due to other toxic effects. Most remarkably, early cooling significantly increased survival in prion-infected mice (91 ± 7 days in cooled mice vs 84 ± 4 days for uncooled mice; P = 0.0002). Indeed, one animal survived 117 days post-infection, nearly a 50% increase in life expectancy (Fig. 3f). Mice cooled later in prion disease, at 5 and 6 w.p.i., when the RBM3 induction response is lost (see Fig. 2b), did not show increased survival (Extended Data Fig. 7). As predicted, RBM3 knockdown by lentivirally mediated RNA interference (RNAi) in the hippocampus abolished the protective effects of early cooling on CA1 pyramidal neurons and spongiform change (Fig. 3e, subpanel iv), on object recognition memory (Extended Data Fig. 6b, c), and on survival (Fig. 3f). As before, misfolded PrP levels were not affected by cooling (Extended Data Fig. 8). In therapeutic human hypothermia, temperatures of ~34°C are used, similar to those of hibernating large mammals such as bears, which are known to induce similar transcriptional changes in RBM3 (ref. 6). Therefore, these physiological changes in small rodents at 16–18°C may well be relevant in therapeutic...
human hypothermia. Cooling of mice to higher core temperature of 26–28 °C, was similarly protective in prion disease, extending survival (Extended Data Fig. 9).

We next asked if RBM3 overexpression alone, in the absence of cooling, was similarly neuroprotective. We overexpressed, or knocked-down, RBM3 in both hippocampi of mice by stereotoxic injection of lentiviruses LV-RBM3 and LV-shRNA-RBM3, respectively. LV-RBM3 produced a threefold increase in RBM3 levels compared to controls up to 8 weeks post-injection; whereas knockdown by LV-shRNA-RBM3 reduced RBM3 levels to 30% of control levels (Fig. 4a). LV-RBM3 treatment, but not LV-control, rescued the early deficit in synapse reassembly in both prion-infected and 5XFAD mice at 6 w.p.i. and 3 months, respectively (Fig. 4b). Furthermore, LV-RBM3 was associated with maintained, supported neuroprotective effects in prion-infected mice: preventing synapse loss (Fig. 4c), synaptic transmission decline (Fig. 4d) and memory and behavioural impairments (Fig. 4e). In vitro, RBM3 has been shown to promote translation22. Increased global protein synthesis rates are profoundly neuroprotective, rescuing synapse number in prion disease12,13,14. We found that RBM3 overexpression rescued levels of global translation, whereas RBM3 knockdown further reduced them, in prion-infected mice at 9 w.p.i. (Fig. 4f) suggesting that this action of RBM3 along with preferential translation of specific RBM3-bound mRNAs, contributes to the synapse regeneration process. LV-RBM3 treatment reduced prion neuropathology and prevented neuronal loss (compare subpanels ii and iii in Fig. 4g) and significantly extended survival of prion-infected animals (Fig. 4h). This was not associated with changes in levels of PrPSc, which were not affected by overexpression of RBM3 (Extended Data Fig. 8). Knockdown of RBM3, in contrast, accelerated synapse loss and memory and behavioural deficits (Fig. 4c–d), accelerating neuronal loss (Fig. 4g, subpanel iv) and significantly shortening survival (Fig. 4h). 5XFAD mice do not allow similar examination of long-term effects of RBM3 overexpression as evolution of deficits and neuronal loss takes many months, and life expectancy is normal. However, RNAi of RBM3 accelerated onset of synaptic loss in 5XFAD mice, which was now seen at 3 months (Extended Data Fig. 10a), suggesting that RBM3 has a long-term protective role in structural plasticity in these mice also. RBM3 knockdown also reduced synapse number and novel object memory in wild-type mice (Extended Data Fig. 10b), thus it is likely to be involved in synaptic maintenance under normal physiological conditions.

In conclusion, we have shown that early synapse loss in mouse models of neurodegenerative disease results, at least in part, from defective synaptic repair processes associated with failure to induce the cold-shock RNA-binding protein, RBM3. This results in impaired synaptic reassembly after cooling, but also appears to be important in the context of protecting against ongoing synaptic toxicity during disease, and in synaptic maintenance in wild-type mice. Our data suggest that further understanding the mechanisms of action of cold-shock proteins such as RBM3 may yield insights into endogenous repair processes and bring new therapeutic targets for neuroprotection in neurodegenerative disease.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** D.P. performed most experimental procedures and analyses. A.B. analysed cold-shock proteins, N.V. and M.G.M. carried out prion inoculations and stereotoxic injections, C.M. performed behavioural tests, J.A.M. and H.R. carried out histological analyses, J.R.S. performed neurophysiological procedures, M.G.M. and T.S. performed ultramicrometry and processed samples for electron microscopy, which was analysed by D.D. A.E.W. provided expertise on cold-shock and protein expression. G.R.M. conceived and directed the project, D.P. and G.R.M. wrote the paper. All authors contributed to discussion and analysis of data and to the final draft of the paper.

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METHODS

Animals. All animal work conformed to UK regulations and institutional guide-
lines and were performed under UK Home Office guidelines.

Prion infection of mice. The 3-week-old tg37 +/− mice11 were inoculated intra-
parenchymally into the right parietal lobe with 30 μl of 1% brain homogenate of Chandler/ RML (Roky Mountain Laboratories) prions under general anaesthetic, as described10. Animals were culled when they developed clinical signs of scrapie as defined in11. Control mice received 1% normal brain homogenate.

5XFAD mice. Founder 5XFAD mice were obtained from the Jackson Laboratory (Bar Harbour, ME, USA). The 5XFAD mice have the following five mutations: Swedish (K670N and M671L), Florida (I71V) and London (Y717V) in human APP695 and human PS1 cDNA (M146I, L286V) under the transcriptional control of the neuron-specific mouse Thy-1 promoter2. Colonies were maintained by crossing hemi-
genic transgenic to wild-type littermates.

Induction of hypothyroidism. FVB, tg37 +/− and 5XFAD mice weighing ≥ 20 g were cooled using 5°-AMP as described16,17, with slight modifications. Freshly prepared 5°-AMP (Sigma) was injected intraperitoneally (0.7 mg per g). Control mice were injected with saline. Mice were maintained at room temperature until core body temperature decreased to 25°C (approximately 60 min). Subsequently, mice were transferred to a refrigerator (5°C) and core body temperature lowered to 16–18°C for 45 min. Mice recovered normal body temperature at room temperature con-
ditions. Cooled samples were collected at the end of the 16–18°C period and rewarmed samples as stated elsewhere in the text.

Electron microscopy data acquisition and analysis of synapse number. Male mice were used to avoid the effects on synapse number of the oestrus cycle. Brains were perfused fixed with 2% glutaraldehyde + 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (final pH 7.3). Slices (300 μm) were cut from the brain and stained with haemotoxylin and eosin in fixed sections of 5 μm thickness. The volume of the pyramidal layer was measured using the Cavalieri estimator (points grid). For neuron mean density slices were stained with NeuN and calculated within an unbiased virtual space. The total number of neurons in CA1 pyramidal layer was estimated with the neuron density and the volume of the hippocampus.

Immunoblotting. Protein samples were isolated from hippocampi using protein lysis buffer (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 1% Na deoxycholate, 0.1% SDS and 125 mM sucrose) supplemented with Phos-STOP and protease inhib-
itors (Complete, Roche), followed by centrifugation and quantification. Protein levels were determined by resolving 20 μg of protein on SDS–polyacrylamide gel electrophoresis gels, transferred onto either nitrocellulose or PDVF membranes and incubated with primary antibodies. Synaptic proteins were detected using the following antibodies: SNAP-25, (1:10,000; catalogue number: aab5666, Abcam), VAMP2 (1:5,000; catalogue number: 104204, Synaptic Systems), NMDA-R1 (1:1,000; catalogue number: G8913, Sigma) and PSD95 (1:1,000; catalogue number: 04-1066, Millipore). Odyssey IRDye800 secondary antibodies (1:5,000; catalogue number: 926-32210/32211 LE-COR) were applied, visualized and quantified using Odyssey infrared imager (Li-COR; software version 3.0). Protein for PrP levels was determined using the primary antibody ICSM35 (1:1,000, catalogue number: 0130-03501, D-GEN). PrP was detected after Protease K digestion. Cold-shock protein levels were determined with antibodies C19P (1:1,000; catalogue number: 10209-2-AP, Proteintech Group, Inc.) and RBM3 (1:500; catalogue number: 14363-1-AP, Proteintech Group, Inc.). Amyloid-β levels in 5XFAD mice were detected by 6E10 antibody (1:1,000; catalogue number: SIG-39320, Covance). Horseradish-
peroxidase-conjugated secondary antibodies (1:10,000; DAKO) were applied and protein visualized using enhanced chemiluminescence (GE Healthcare) and quanti-
tified using ImageJ. An antibody against GAPDH (1:15,000; catalogue number: sc32233, Santa Cruz) was used to determine gel loading.

Lentiviruses. GenTarget (San Diego, CA, USA) generated lentiviral plasmids. The neuron-specific promoter CAMKII was used to drive RBM3; the H1 promoter was used for shRNA-RBM3 expression and scrambled sequence-shRNA. Viruses were injected stereotaxically into the CA1 region of the hippocampus as described19.

Mouse RBM3 isoform 2 (NM_001166410.1) overexpression was induced using the pLentiCAMKII (RB3M) (RFPbsd) plasmid. pLentiCAMKII (empty)Rv (RFPbsd) was used as control. RBM3 down regulation was achieved by using pLentIH1 shRNA (mRB3M) sequence number 2Rv (RFPbsd). This plasmid con-
tains the following shRNA-RBM3 sense, anti-sense and loop sequences (sequence number 2: 5’-GTCTGATCTGAGGAAAGGTCTcagAGCATTTTGCTCGATAG TCGATCG-3’).

pLentIH1 FabRNA (negative control)Rv (RFPbsd) containing the sequence 5’- GTCCACGGGCACGTATT3-3’ was used as control. Lentiviral sequences and viral stocks were generated by GenTarget (San Diego, CA, USA). Virus titre was determined using FACS (BD FACs Calibur). Viruses were used with a final titre of 6.0×10^5 to 1.0×10^6 transducing units.

Stereotaxic injection. Under general anaesthetic, mice were injected with 5 μl of lentivirus per site into the CA1 region of the hippocampus. Mice were injected at 2 locations per hemisphere; at −2 mm and −2.7 mm posterior, ±2 mm lateral and −2.2 mm ventral relative to bregma, using a 26 g-sauge needle and Hamilton syringe as described20.

Burrowing assay. This was performed as described15,16. Briefly, mice were placed in individual large plastic cages containing a clear Perspex tube, 20 cm long × 6.8 cm diameter, filled with 140 g of normal food pellets. The weight of pellets remaining in the tube was measured after 2 h and the percentage burrowed calculated. Behavioural data were analysed using one-way ANOVA with Brown–Forsythe test and Tukey’s post hoc test. For behavioural testing no formal randomization was needed or used. Experimenter was blind to group allocation during all experiments and when assessing outcome.

Novel object recognition memory. This was performed as described13. Briefly, mice were tested in a black cylindrical arena (69 cm diameter) mounted with a 100 LFD (30 W) red light-emitting diode (LED) and a 70 cm diameter video camera (Sony). Mice were acclimatized to the arena 5 days before testing. During the learning phase, two identical objects were placed 15 cm from the sides of the arena. Each mouse was placed in the arena by an operator blind to the experimental group for two blocks of 10 min for exploration of the objects with an inter-trial interval of 10 min.
Two hours later, one of the objects was exchanged for a novel one, and the mouse was re-introduced to the arena for 5 min (test phase). The amount of time spent exploring all objects was tracked and measured for each animal using Ethovision software (Tracksys). All objects and the arena were cleaned thoroughly between trials to ensure the absence of olfactory cues. The amount of time spent exploring the novel object over the familiar object is expressed as a ratio, where a ratio of 1 reflects random exploration, and >1 reflects memory. Behavioural data were analysed using one-way ANOVA with Brown–Forsythe test and Tukey’s post hoc test. For behavioural testing no formal randomization was needed or used. Experimenter was blind to group allocation during all experiments and when assessing outcome.

Electrophysiology. Whole-cell recordings were made in acute hippocampal slices of CA1 neurons and recording performed as described. In brief, neurons were voltage clamped using a Multiclamp 700B amplifier and pClamp 10.3 software (Molecular Devices) and EPSCs were evoked by stimulation with bipolar platinum electrode at 37 °C. Pipettes (2.5–3.5 MΩ) were filled with a solution containing (in mM): KCl 110, HEPES 40, EGTA 0.2, MgCl₂ 1, CaCl₂ 0.1; pH was adjusted to 7.2 with KOH. Neurons were visualized with ×60 objective lenses on a Nikon FS600 microscope fitted with differential interference contrast optics. Four to eight cells were measured per mouse in at least two animals per experiment. Male mice were used to avoid effects of the oestrus cycle.

Hippocampal slice preparation and ³⁵S-methionine labelling. Slices were dissected in an oxygenated cold (2–5 °C) sucrose artificial cerebrospinal fluid (ACSF) containing (in mM): 26 mM NaHCO₃, 2.5 mM KCl, 4 mM MgCl₂, 0.1 mM CaCl₂, and 25 mM sucrose. Hippocampal slices were prepared using a tissue chopper (McIlwain). Slices were allowed to recover in normal ACSF buffer while being oxygenated at 37 °C for 1 h, then incubated with [³⁵S]-methionine label for 1 h, then homogenized. Proteins were TCA precipitated and incorporation of radiolabel was measured by scintillation counting (Winspectal, Wallac).

Statistics. Statistical analyses were performed using Prism v5 software, using Student’s t-test for data sets with normal distribution and a single intervention; when the F-test to compare variances was significant, Mann–Whitney U-test was performed instead.

Behavioural data, neuronal counts and ³⁵S-met were analysed using one-way ANOVA and Tukey’s post hoc test for multiple variables. For behavioural testing no formal randomization was needed or used. Experimenter was blind to group allocation during the experiments and when assessing outcome.

Statistical analyses for in vivo experiments. Sample size estimation for induction of hypothermia for volume, synaptic density and estimation of total number of synapses was based in effect size of the preliminary experiment on dissector method (5.9511) and obtained with the free software G*Power version 3.1.9.2.

The software prediction shows that with a sample size of 6 animals for 2 conditions (control and cooled or cooled and rewarmed), the experiment has a 99.8% chance of detecting a difference and avoid a type II error (β-error), with a 0.05% chance of a type I (α-error). Sample size estimation for novel object recognition experiment was established based on the effect size of 1.6161 from control and prion mice at 8 w.p.i. This parameter was applied in the following F tests calculation of power analysis with G*Power version 3.1.9.2.

Similar analyses were performed for burrowing tests.

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Extended Data Figure 1 | Stereological assessment of volume and synapse size to validate 2D assumption-based approaches for counting synapse density. a, CA1 volume and synapse mean length and area in the stratum radiatum remain essentially unchanged on cooling and rewarming in wild-type mice. Volume was measured using disector principle and synapse mean length and area determined in the same sections, as described, \( n \) values as reported for Fig. 1a. b, c, Representative electron micrographs (pseudo-coloured for ease of synapse identification) for data not shown in Fig. 1b, c, from prion-infected mice at 4 and 6 w.p.i. (b) and for 5xFAD mice at 2 and 3 months (c) before cooling (black framed images) and cooled (blue framed images). d, Schematic showing lost capacity for structural plasticity precedes synapse loss and neuronal loss in both mouse models. Scale bar, 1 \( \mu \)m. All data in bar charts are mean \( \pm \) s.e.m. Student’s \( t \)-test, two tailed. Non-significant \( P \) values.
Extended Data Figure 2 | Synaptic protein levels during cooling–rewarming in prion and 5xFAD mice. a, Levels of presynaptic (SNAP25, VAMP2) and postsynaptic (PSD95, NR1) proteins do not change before (black bars) and after cooling to 16–18 °C (blue bars) in prion-infected mice at 4 and 6 w.p.i., b, 5xFAD mice at 2 and 3 months. Representative western blots are shown for 3 mice per temperature and time point. Bar graphs show quantification of synaptic protein levels relative to GAPDH. All data represent means ± s.e.m. (n = 3–11 mice per time point). Student’s t-test, two tailed. n.s. = non-significant P values.
Extended Data Figure 3 | Cooling does not induce changes in PrPSc or amyloid-β levels. a, Levels of total PrP (upper blot) and PrPSc (lower blot) do not change notably before (white line), during (blue line) or after (red line) cooling to 16–18°C in prion-infected mice. PrPSc is detected after digestion with proteinase K. Levels are undetectable by western blotting at 6 w.p.i., as expected. b, Cooling does not change levels of amyloid-β oligomers in 5xFAD mice, arrow indicates amyloid-β monomers (lane 1, synthetic amyloid-β oligomers; last lane, one-year-old 5xFAD control (C+)). Representative western blots are shown for 3 mice per temperature and time point. Non-significant P values.
Extended Data Figure 4 | Cooling induces sustained increase in RBM3 levels but not in CIRP. a, b, Levels of CIRP do not change after cooling in prion-infected mice at 4 and 6 w.p.i. (a) or in 5xFAD mice at 2 and 3 months (b). Representative western blots are shown for 3 mice per temperature and time point. Bar graphs show quantification of CIRP levels relative to GAPDH. All data represent means ± s.e.m. (n = 6–9 mice per time point). Student’s t-test, two tailed. n.s. = non-significant P values. c, Increased levels of RBM3 are sustained for at least 72 h after cooling in wild-type mice. Bar graph shows quantification of RBM3 against GAPDH in control (white bar), cooled (blue bar), and 12, 48 and 72 h recovery after cooling (red bars). All data represent means ± s.e.m. (n = 3–6 mice per time points, *P < 0.05, Mann–Whitney U-test, two tailed).
Extended Data Figure 5 | Early cooling induces sustained elevation of RBM3 levels. RBM3 levels remain high after cooling to 16–18 °C in prion-infected mice (magenta boxes) compared to control prion-infected mice. These levels remained high up to 6 weeks later and declined at 12 w.p.i. Representative western blots are shown for 3 mice per time point.
Extended Data Figure 6 | Exploration time in exposure phase of novel object testing is normal in all groups and RBM3 knockdown abolishes improved memory after cooling. a, Exploratory behaviour measured in seconds is not different in mice with early cooling from prion-diseased mice and is not affected by the duration of disease (n as reported in Fig. 3d). b, c, Lentivirally mediated RNAi of RBM3 eliminates the protective effect of cooling on novel object memory impairment in prion disease (b) (dark green bar); but does not affect exploratory behaviour in training phase (c). All data represent means ± s.e.m. Data analysed using one way ANOVA, Brown–Forsythe test with Tukey’s post hoc analysis for multiple comparisons (n = 11–16 mice per time point, **P < 0.01).
Extended Data Figure 7 | Induction of hypothermia at time point when 
RBM3 induction fails is not neuroprotective. Cooling at 5 and 6 w.p.i., when 
synaptic plasticity and RBM3 induction fails (see Fig. 1 and 2, main text), does 
not increase survival in prion-infected mice. Kaplan–Meier survival plots for 
prion-infected mice (black line, no cooling; n = 10; orange line, mice cooled at 
5 and 6 w.p.i., n = 16). Student’s t-test, two tailed. Non-significant P values.
Extended Data Figure 8 | PrPSc levels remain unchanged in prion with overexpression of RBM3. a, b, In prion-infected mice total PrP and PrPSc levels do not alter after early cooling to 16–18 °C (a) (magenta boxes) or following treatment with LV-RBM3 (dark green) and LV-shRNA-RBM3 (pale green) (b). PrP and PrPSc levels tested in 9 w.p.i. and terminal mice. PrPSc is detected after digestion with proteinase K. Representative western blots are shown for 3 mice per temperature and time point, the lane marked C shows uninfected control mouse.
Extended Data Figure 9 | Mild hypothermia also extends survival in prion-infected mice. Kaplan–Meier plot showing that cooling to 26 °C at an early stage also significantly lengthens survival (n = 27 cooled vs n = 16 non-cooled mice); **P < 0.01, Student's t-test, two tailed.
Extended Data Figure 10 | RNAi of RBM3 downregulation accelerates impaired structural synaptic plasticity in the 5xFAD mouse model, and also reduced synapse number and function in wild-type mice. a, Impaired structural synaptic plasticity after cooling occurs in shRNA-RBM3 treated 5xFAD mice at 3 months. Representative electron micrographs are shown and are pseudo-coloured as in main text figures. Quantification shows significant reduction in synapse number by RNAi of RBM3 (n = 82–93 images from 3 mice per time point, Student’s t-test, two tailed). b, RBM3 knockdown reduces synapse number and novel object memory in wild-type mice (n = 93 images from 3 mice per time point, Student’s t-test, two tailed ***P < 0.0001; for novel object recognition task n = 11 mice, LV-shRNA-control and 10 mice, LV-shRNA-RBM3, Mann–Whitney U-test, *P < 0.05). Scale bar, 1 µm.