tested by ANOVA ($P < 0.05$)[10,29]. A correlation coefficient between top-down and bottom-up responses was calculated for each single neuron. We performed Monte Carlo simulation by randomizing the assignment of cue stimulus and by recalculating the correlation for 10,000 times in each neuron to estimate a baseline correlation level. The median values of 10,000 simulated correlation coefficients ranged from −0.007 to +0.005 ($n = 43$ cells). Category selectivity was appraised using ANOVA with mean cue responses in each category used as units of analysis and *post-hoc* multiple comparisons (Tukey's method), evaluated at $P = 0.05$. This procedure correctly rejects the case in which neurons are strongly activated only by a single stimulus. Error trials were omitted for the analysis of the choice responses. Trial based instantaneous firing rate (IFR) was defined as the mean discharge rate during the 100-ms window centred at the given time point stepped by 10 ms. Averaging the IFRs first across trials for each cue, then across cues in each category resulted in mean IFRs of five categories.

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L-type calcium channels and GSK-3 regulate the activity of NF-ATc4 in hippocampal neurons

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The molecular basis of learning and memory has been the object of several recent advances, which have focused attention on calcium-regulated pathways controlling transcription. One of the molecules implicated by pharmacological, biochemical and genetic approaches is the calcium/calmodulin-regulated phosphatase, calcineurin[1–5]. In lymphocytes, calcineurin responds to specific calcium signals and regulates expression of several immediate early genes by controlling the nuclear import of the NF-ATc family of transcription factors[6–9]. Here we show that NF-ATc4/NF-AT3 (ref. 10) in hippocampal neurons can rapidly translocate from cytoplasm to nucleus and activate NF-AT-dependent transcription in response to electrical activity or potassium depolarization. The calcineurin-mediated translocation is critically dependent on calcium entry through L-type voltage-gated calcium channels. GSK-3 can phosphorylate NF-ATc4, promoting its export from the nucleus and antagonizing NF-ATc4-dependent transcription. Furthermore, we show that induction of the inositol 1,4,5-trisphosphate receptor type 1 is controlled by the calcium/calcineurin/NF-ATc pathway. This provides a new perspective on the function of calcineurin in the central nervous system and indicates that NF-AT-mediated gene expression may be involved in the induction of hippocampal synaptic plasticity and memory formation.

NF-ATc4 messenger RNA was observed in both murine and rat hippocampus using *in situ* hybridization (Fig. 1a) and polymerase chain reaction with reverse transcriptase (RT-PCR) (Fig. 1b). We tested whether endogenous NF-ATc proteins in neurons would activate NF-AT-dependent transcription by transfecting cultured hippocampal neurons with a reporter plasmid in which NF-AT-binding sites control expression of green fluorescent protein (GFP). This strategy allowed us to test the activity of the endogenous NF-AT transcription complex, which in other cell types is controlled through calcineurin and ras signalling[6,11]. Cotransfection of constitutively active calcineurin and V12 Ras strongly promoted the expression of the reporter in pyramidal neurons (Fig. 1c). NF-AT-dependent transcription was quantified with a luciferase reporter construct[8] (Fig. 1d). Three-hour stimulation with the calcium ionophore ionomycin and phorbol-12-myristate-13-acetate (PMA) activated luciferase expression driven by endogenous NF-AT. This activity was blocked by a combination of the calcineurin inhibitors FK506 and cyclosporin A (CsA) (Fig. 1d). The high level of calcineurin expression in hippocampal pyramidal neurons led us to use both drugs. The concentrations of FK506 and CsA used in these studies did not inhibit the expression of a constitutively active luciferase reporter gene (data not shown). The NF-AT reporter was dependent upon endogenous neuronal NF-AT, because deletion of the trimerized NF-AT-binding sites abolished activity (Fig. 1d).

Spontaneous electrical activity was a powerful activator of NF-AT-dependent transcription in hippocampal neurons that had

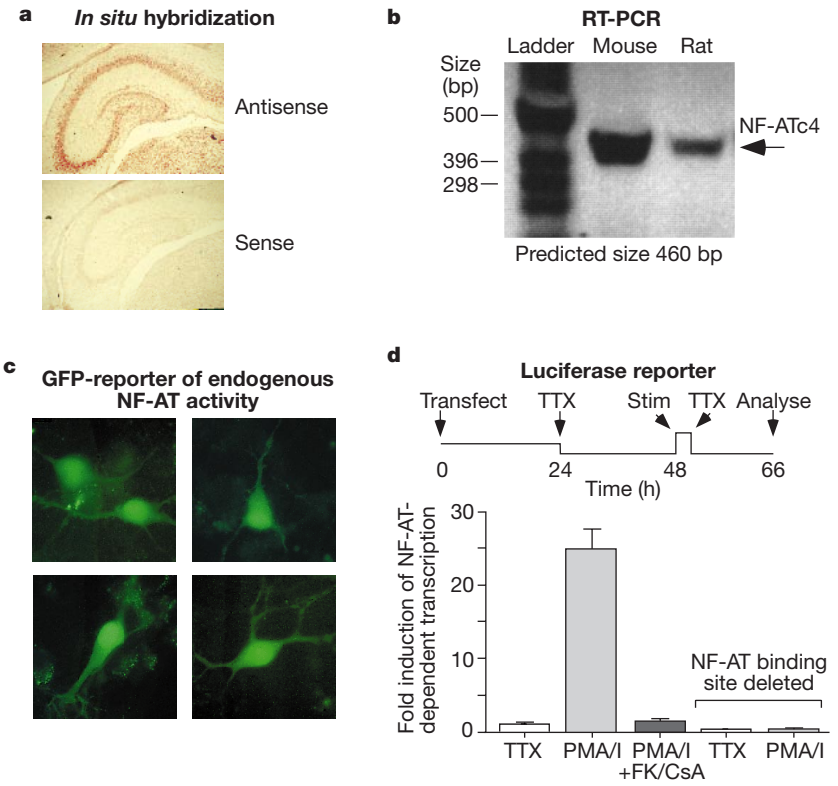

Figure 1 Expression of NF-ATc4 and activation of NF-AT-dependent transcription by endogenous NF-AT proteins in the hippocampus. **a**, *In situ* hybridization of NF-ATc4 messenger RNA in rat hippocampus. **b**, RT-PCR showing NF-ATc4 mRNA in the murine and rat hippocampus. **c**, Cotransfection of constitutively active calcineurin (0.5 μg) and V12-ras (2 μg) activated endogenous NF-AT proteins and thereby the expression of an NF-AT-dependent reporter construct (4 μg) driving the expression of GFP in pyramidal neurons. **d**, Activation of endogenous NF-AT protein leads to expression of NF-AT-dependent luciferase (4 μg) after pharmacological stimulation with phorbol-12-myristate-13-acetate (PMA, 25 ng ml^{-1}) and ionomycin (I, 1 μM) and was blocked by addition of FK506 (200 ng ml^{-1}) and CsA (1 μg ml^{-1}). A mutant NF-AT-luciferase reporter construct with the trimerized NF-AT binding sites deleted from the minimal IL-2 promotor is no longer activated by PMA and I stimulation.

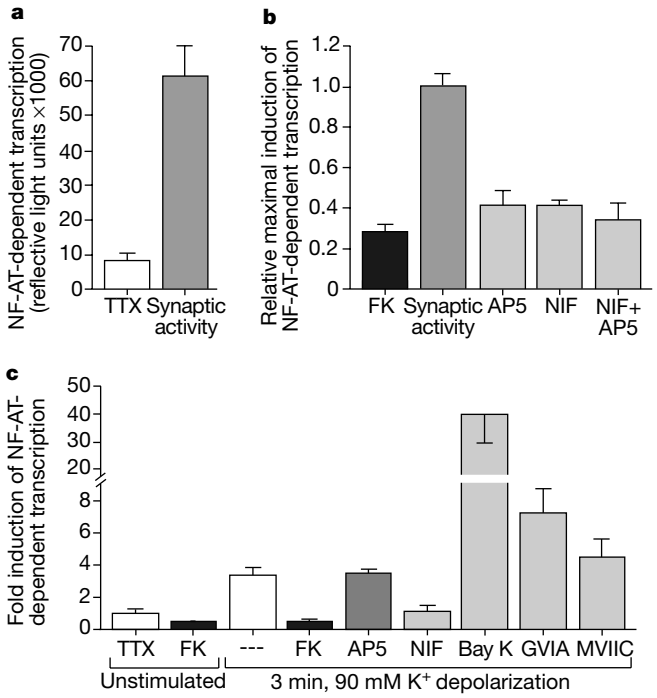

Figure 2 Activation of NF-AT-dependent transcription by NMDA receptors and L-type Ca^{2+} channels. **a**, TTX blocked the activation of NF-AT-dependent transcription by endogenous NF-AT proteins in hippocampal neurons transfected with an NF-AT-luciferase reporter plasmid (4 μg) at 9 days *in vitro*, a time when synaptic connections had developed[30]. **b**, FK506/CsA, the L-type channel blocker nifedipine (5 μM) and AP-5 (25 μM) inhibit NF-AT-dependent luciferase activity induced by spontaneous electrical activity. **c**, Three minute, 90 mM K^+ depolarization activated NF-AT-dependent luciferase expression. Transcription was blocked by FK506 and nifedipine, but not by AP-5. (–)-Bay K8644 (5 μM) strongly augmented the transcriptional response. NF-AT–luciferase activity was not blocked by the ω-CgTx-GVIA (1 μM) or ω-CTx-MVIIC (5 μM). For the experiments in Fig. 2c, NF-AT–luciferase (4 μg) and EGFP-NF-ATc4 (1 μg) were cotransfected.

developed synaptic connections (Fig. 2a). Inhibition of action potentials with the Na^+-channel blocker tetrodotoxin (TTX) (Fig. 2a) or inhibition of calcineurin by FK506/CsA (Fig. 2b) markedly reduced NF-AT-directed gene expression. To determine whether synaptic activity was required, we applied pharmacological blockers of key postsynaptic neurotransmitter receptors and voltage-gated Ca^{2+} channels (Fig. 2b). The NMDA (N-methyl-D-aspartate)-receptor antagonist AP-5 (25 μM) and the L-type Ca^{2+} channel blocker nifedipine (5 μM) each reduced NF-AT-dependent gene expression to an extent not significantly different from the blockers in combination (Fig. 2b). Evidently, activity of both kinds of channels was critical under these circumstances.

We further explored Ca^{2+} entry mechanisms linked to NF-ATc4 activation using direct depolarization of hippocampal neurons (Fig. 2c). A 3-min exposure to 90 mM K^+ resulted in activation of NF-AT-dependent gene expression. The K^+-induced increase in NF-AT-dependent transcription was blocked by nifedipine or FK506, but not by AP-5 (Fig. 2c). Pre-exposure to the L-type Ca^{2+} channel agonist (−)-Bay K8644 (5 μM) strongly augmented the NF-AT transcriptional response to K^+ depolarization. In contrast to the clear participation of L-type channels, blockade of N-type Ca^{2+} channels by ω-CTx-GVIA (1 μM) or P/Q- and N-type channels with ω-CTx-MVIIC (5 μM) did not reduce the transcriptional response (Fig. 2c). Thus, the K^+ depolarization acts specifically through L-type Ca^{2+} channels to induce NF-AT-dependent transcription. During the course of spontaneous electrical activity (Fig. 2b), activation of L-type channels is driven by synaptic transmission through NMDA and other glutamate receptors.

In lymphocytes, Ca^{2+} entry regulates the activity of calcineurin, a Ca^{2+}/calmodulin-dependent phosphatase that dephosphorylates NF-ATc family members[9,12], unmasking their nuclear localization sequences and leading to nuclear import[13]. To determine whether nuclear translocation of NF-ATc4 takes place in neurons, we monitored cellular localization of enhanced green fluorescent protein (EGFP)-NF-ATc4 with fluorescence imaging (Fig. 3). K^+ depolarizations produced a rapid redistribution of EGFP-NF-ATc4, monitored with real time imaging of GFP (data not shown). Nuclear NF-ATc4 levels were greatly increased 15 min after the 3-min depolarization and returned to baseline after 2 h (Fig. 3a, b). The persistence of NF-ATc4 in the nucleus of hippocampal neurons following a brief Ca^{2+} pulse is in sharp contrast to lymphocytes, where a sustained elevation of intracellular Ca^{2+} is required to maintain NF-ATc proteins in the nucleus[14]. This difference in kinetics might be fundamental to the requirement for rapid responses to Ca^{2+} in neurons. The increase in nuclear-to-cytoplasmic ratio was blocked by the L-type channel antagonist nifedipine and by inhibition of calcineurin with FK506/CsA (Fig. 3a, b). Depolarization in the presence of Bay K8644 produced no greater increase in nuclear-to-cytoplasmic ratio but retarded exit from the nucleus over the 2 h following stimulation (Fig. 3a, b). This altered time course is consistent with the powerful effect of the L-type channel agonist on reporter gene activation (Fig. 2c). Electrical field stimulation (5 Hz for 3 min) and spontaneous electrical activity also induced a strong increase in the level of nuclear NF-ATc4 (Fig. 3c, d), consistent with the effect of electrical activity on NF-AT-dependent transcription (Fig. 2a). Direct stimulation of NMDA receptors (10 μM NMDA for 3 min) also induced nuclear translocation of NF-ATc4 which was dependent on L-type channels (Fig. 3d). Longer NMDA stimulation (15 min) caused more pronounced nuclear translocation of NF-ATc4, but it too was

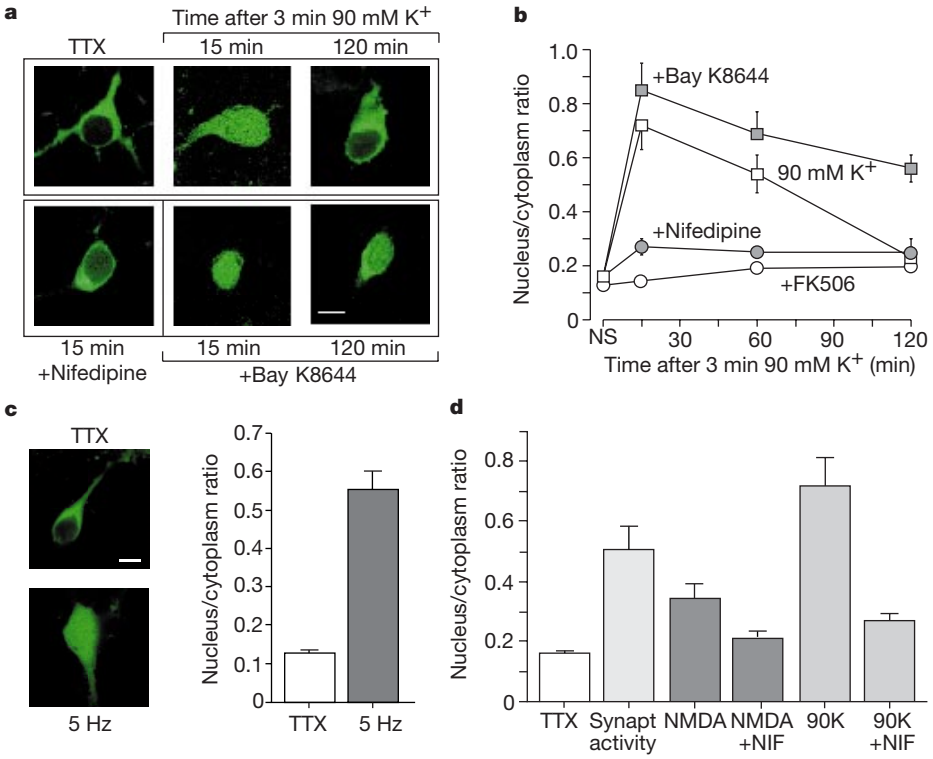

Figure 3 Activity-dependent cytoplasmic-to-nuclear translocation of NF-ATc4 in hippocampal neurons. Cells were transfected with EGFP-NF-ATc4 plasmid (4 μg) for all imaging experiments. **a**, Confocal sections showing NF-ATc4 distribution following 3 min 90 mM K^+ depolarization in the absence and presence of nifedipine or Bay K8644. **b**, Confocal quantification of EGFP-NF-ATc4 translocation after 3 min K^+ stimulation. Nifedipine and FK506/CsA inhibited nuclear import of NF-ATc4, whereas Bay K8644 slowed its nuclear export. **c**, Nuclear translocation of EGFP-NF-ATc4 upon synaptic stimulation. Confocal images and quantification of EGFP-NF-ATc4 translocation upon electrical field stimulation at 5 Hz for 3 min. **d**, Quantification of EGFP-NF-ATc4 translocation in response to spontaneous synaptic activity and direct 3 min NMDA-receptor stimulation and 3 min K^+ stimulation. Nifedipine inhibited the nuclear translocation of NF-ATc4 induced by NMDA-receptor stimulation.

blocked by nifedipine. These results show that L-type Ca^{2+} channels increase nuclear NF-ATc4, thus activating NF-AT-dependent transcription.

As the rate of nuclear efflux of EGFP-NF-ATc4 in hippocampal neurons could be modulated by L-type channel agonists (Fig. 3a, b), we considered possible export mechanisms. In nonexcitable cells, bidirectional shuttling of NF-ATc proteins between cytoplasm and nucleus is regulated by calcineurin and opposing kinases. Several Ser/Thr kinases, including GSK-3β[15], JNK[16], MEKK[17] and casein kinase 1α[17] phosphorylate NF-ATc proteins at specific amino-terminal motifs[13], thereby opposing the actions of calcineurin. The effects of these kinases in neurons were tested by cotransfection with the NF-AT luciferase reporter. Overexpression of GSK-3β inhibited the activation of NF-AT-dependent transcription in response to PMA plus ionomycin treatment and K^+ depolarization (Fig. 4a), but had no effect on the expression of a constitutively active reporter gene (data not shown). In contrast to GSK-3β, cotransfection of JNK, constitutively active MEKK or casein kinase 1α produced no inhibitory effect (Fig. 4b). The physiological importance of endogenous GSK-3 was examined by expression of a dominant negative form of GSK-3β[18], which augmented the activation of the NF-AT-dependent reporter gene (Fig. 4c); GSK-3 was present in the nuclear fraction of lysed hippocampal neurons as determined by subcellular fractionation and western blotting (Fig. 4d). Accordingly, we examined the *in vitro* phosphorylation of an NF-ATc4–GST fusion protein by hippocampal extracts and found four distinct phosphorylation bands (Fig. 4e). To test whether any of these were due to endogenous GSK-3, we used antibodies specific for GSK-3α and β to immunodeplete the extracts. The GSK-3-depleted extracts failed to produce two of the four phosphorylated bands (middle lane, Fig. 4e), in contrast to extracts mock-depleted with protein-G beads, which produced all four phosphorylation bands (left lane, Fig. 4e). Recognition of substrates by GSK-3 usually requires prior phosphorylation of the substrate by a priming kinase. Using NF-ATc4 that had been treated with GSK-3α and β-depleted extracts to provide this priming phosphorylation, we found that recombinant GSK-3β produced two phosphorylation bands with the same mobilities as the bands that had been eliminated by GSK-3 depletion (right lane, Fig. 4e).

We devised a nuclear export assay to assess the effects of GSK-3β on nuclear export of EGFP-labelled NF-ATc4 in hippocampal neurons. Transfection of neurons with constitutively active calcineurin established a stable starting condition with EGFP-NF-ATc4 abundant in the nucleus (Fig. 4f, g). Nuclear exit of the fluorescently tagged transcription factor was initiated by blocking calcineurin activity with FK506 and CsA, and monitored with measurements of the nuclear-to-cytoplasmic ratio of EGFP fluorescence. Neurons transfected with GSK-3β showed more than 3-fold faster export than those transfected with empty vector (Fig. 4f, g). Expression of a dominant negative form of GSK-3β slowed the rate of nuclear export of NF-ATc4 (Fig. 4g), consistent with its effect on the transcriptional response (Fig. 4c). Thus GSK-3β acts in hippocampal nuclei to phosphorylate NF-ATc4, thereby directing its nuclear export and terminating transcription.

The inositol 1,4,5-triphosphate receptor type 1 (IP_3R1) is found throughout the endoplasmic reticulum network in neurons and is a major determinant of intracellular Ca^{2+} transients. In cerebellar granule cells, transcription of the IP_3R1 gene is regulated by Ca^{2+} entry through L-type channels and is inhibited by FK506 and CsA, implicating calcineurin as a regulator[19]. Likewise, in hippocampal neurons, K^+ depolarization in the presence of Bay K8644 caused a substantial increase in IP_3R1 protein levels relative to those observed in neurons stimulated in the presence of FK506 and CsA (Fig. 5a). In

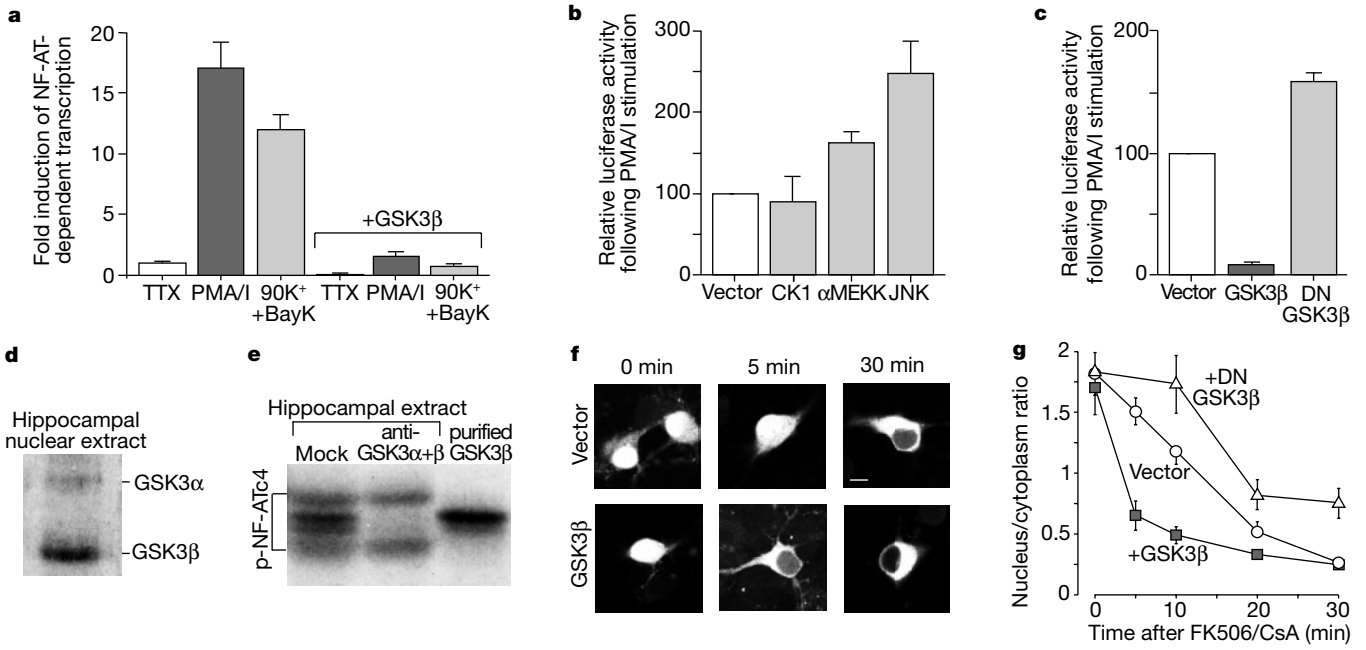

Figure 4 GSK-3β blocks NF-AT-dependent transcription and promotes nuclear export of NF-ATc4. **a**, Overexpression of GSK-3β inhibited activation of NF-AT-dependent transcription. Neurons were cotransfected with plasmids for GSK-3β (4 μg) or empty vector (4 μg), along with NF-AT-luciferase (4 μg) and pBJ5-NF-ATc4 (0.5 μg). **b**, Cotransfection of JNK-1 (4 μg), constitutively active MEKK (4 μg) and casein kinase 1α (4 μg) produced no inhibitory effect upon NF-AT-dependent luciferase expression. **c**, Cotransfection of dominant negative GSK-3β (4 μg) augmented the activation of NF-AT-dependent transcription. **d**, Immunoblot showing GSK-3α and β in the nucleus of hippocampal neurons. **e**, Endogenous GSK-3 activity in the hippocampus phosphorylated NF-ATc4. **f**, Accelerated nuclear export of NF-ATc4 after overexpression of GSK-3β, shown in representative confocal sections. Neurons were cotransfected with EGFP-NF-ATc4 (2 μg), constitutively active calcineurin (0.5 μg) and GSK-3β (4 μg) or empty vector (4 μg). FK506 (400 ng ml^{-1}) and CsA (2 μg ml^{-1}) were added to inhibit calcineurin at 0 min. **g**, Quantification of NF-ATc4 export kinetics in the presence of empty vector (4 μg), GSK-3β (4 μg) and dominant negative GSK-3β (4 μ) and dominant negative GSK-3β (4 μg) after FK506/CsA addition.

preliminary studies, we found that mice with null mutations of NF-ATc4 or mice with null mutations in NF-ATc2 and NF-ATc4 have low levels of expression of the IP_3R1 gene (I.G. and G.C., unpublished data). The 5′ flanking region of the murine IP_3R1 gene contains many candidate NF-AT binding sequences, four of which were chosen for further study (Fig. 5b).

Nuclear extracts from rat hippocampi contained binding activity for the NF-AT consensus sequence (CGGGGAAAGTTTGT) from the IP_3R1 5′ flanking sequence (Fig. 5c). Binding to this probe was partially competed with an excess of unlabelled oligonucleotide from the distal NF-AT-binding site from the interleukin (IL)-2 gene (ARRE)[8], and with the unlabelled IP_3R1 site (Fig. 5c). Recombinant NF-ATc4, prepared from transfected Jurkat cells stimulated with PMA and ionomycin (Fig. 5c), produced a DNA–protein complex with the IP_3R1 probe similar to that seen with extracts from hippocampal neurons.

A property of NF-AT transcription complexes[6,8] is their ability to integrate signalling pathways[20] by the combined action of NF-ATc and a nuclear partner protein, NF-ATn[21]. To see whether this was true for the IP_3R1 site, we performed a mixing experiment similar to that used to define the original NF-AT1 transcription complex[6]. By themselves, extracts of nonstimulated hippocampal cells and extracts of PMA-treated JST-cells contained little DNA-binding activity; however, the two extracts in combination produced a substantial enhancement of DNA-binding activity (Fig. 5d). Thus, the IP_3R1 NF-AT consensus sequence has the properties of a functional NF-AT site. We tested whether the nuclear partner protein might be AP-1, as found in lymphocytes[12]. There are no consensus AP-1 sites surrounding the neuronal IP_3R1 NF-AT elements, and an unlabelled AP-1-binding oligonucleotide failed to compete with binding of hippocampal extracts to the IP_3R1 NF-AT site (Fig. 5c, lane 4). This indicates that the nuclear partner of NF-ATc4 in neurons may be a binding protein distinct from AP-1.

The interplay between the regulated nuclear import and export of NF-ATc4 that we have found in hippocampal neurons represents a novel mechanism by which neuronal Ca^{2+} signals may control gene expression (Fig. 5e). Synaptic activity or direct depolarization produced rapid translocation of NF-ATc4 to the nucleus and NF-AT-dependent gene expression, showing that this pathway can function in parallel with other pathways for transcriptional control by Ca^{2+} (refs 22, 23). The regulation of IP_3R1 expression may have interesting implications for forms of synaptic plasticity such as long-term depression, whose induction in the hippocampus and cerebellum depends on Ca^{2+} release from IP_3-sensitive stores[24–26]. The NF-AT-dependent induction of IP_3R1 could alter the amplitude and/or spatial organization of the Ca^{2+} signal in neurons and would be well suited as a feedback mechanism for stabilization of changes in synaptic strength, which is interesting because transcription and translation are necessary for long-term memory formation[27,28]. □

Methods

In situ hybridization and RT-PCR

Hybridization with a digoxigenin-labelled riboprobe (Boehringer Mannheim) was performed using standard methods. The NF-ATc4 probe was an *Eco*RI/*Bam*HI fragment containing part of exon 2 of the murine NF-ATc4 gene. RT-PCR, using cDNA prepared from murine and newborn rat hippocampus, was performed according to standard protocols. The primers used were derived from exon 2 and exon 3 of the murine NF-ATc4 gene (5′-GAAGCTACCCTCCGGTACAGAG-3′ and 5′-GCTTCATAGCTGGCTG-TAGCC-3′).

Cell culture and transfections

Cultured CA3/CA1 hippocampal neurons were transfected using a Ca^{2+}-phosphate procedure at 7 days *in vitro*[29]. For luciferase assays cell media contained 1–2 μM

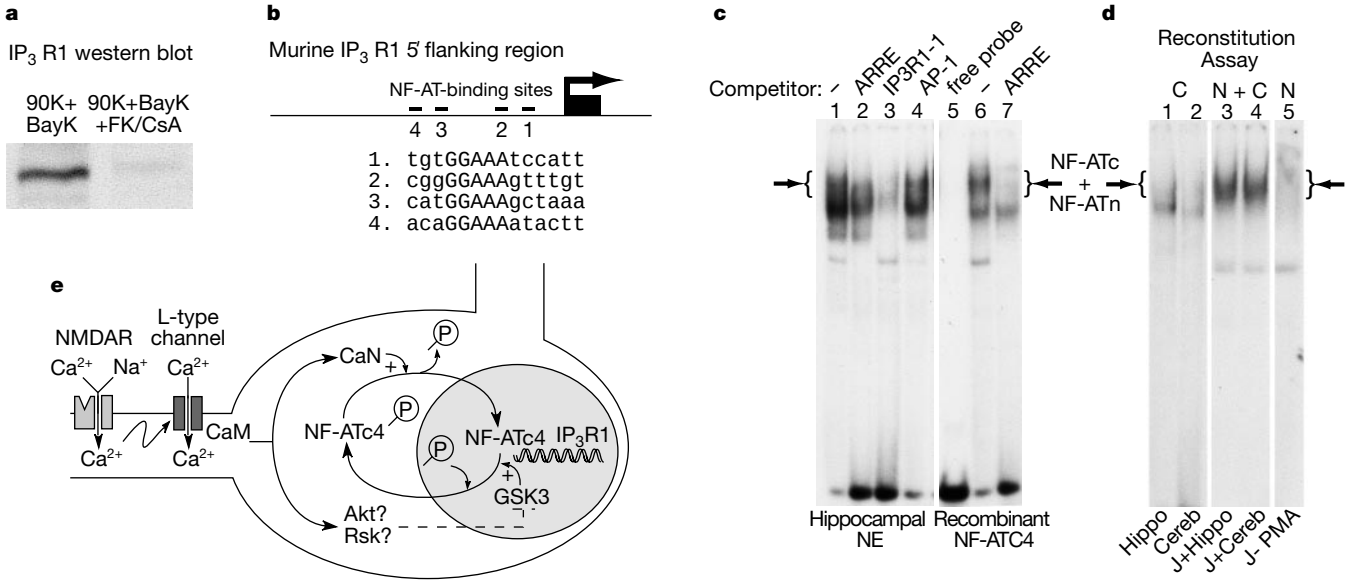

Figure 5 NF-AT-dependent regulation of IP_3R1. **a**, IP_3R1 protein expression is regulated by calcineurin in hippocampal neurons. Western blot showing inhibition by FK506 ICsA of IP_3R1 protein expression induced by L-type channels. **b**, Schematic of the 5′ flanking region of the murine IP_3R1 gene. The four indicated NF-AT consensus sites were analysed for NF-AT binding by gel mobility shift assay. **c**, NF-AT in hippocampal nuclear extracts gives rise to specific DNA-binding activity with the IP_3R1 NF-AT consensus site oligonucleotide. A ^{32}P-labelled oligonucleotide from the putative IP_3R1 NF-AT-binding site 2 was used in gel mobility shift assays with nuclear extracts from rat hippocampi (5 μg) complemented with nuclear extract from PMA-stimulated JST cells (lane 1–4) (2.5 μg). Cold competitor oligonucleotides are indicated and were used at 100-fold excess. Arrows indicate specific NF-AT complexes. Nuclear extracts from NF-ATc4 transfected Jurkat T-cells stimulated with PMA Ionomycin (5 μg) bound to the IP_3R1 probe (lane 6), and the binding was competed by an excess of cold ARRE oligonucleotide (lane 7). Lane 5 shows the free IP_3R1 NF-AT-binding site 2 probe. NE, nuclear extracts. **d**, Cooperative DNA binding to the IP_3R1 probe of NF-ATc from hippocampal and cerebellar extracts and NF-ATn from PMA-stimulated nuclear extracts. Binding reactions were carried out with NF-ATc from hippocampal extracts (10 μg) or cerebellar extracts (10 μg) (lanes 1 and 2), or hippocampal and cerebellar extracts (10 μg) combined with nuclear extract (5 μg) from PMA-stimulated JST cells (lanes 3 and 4), or nuclear extract from PMA stimulated JST-cells (5 μg) alone (lane 5). **e**, Interplay of dephosphorylation and phosphorylation in the regulation of NF-ATc4 in hippocampal neurons.

tetrodotoxin (TTX; Calbiochem) after transfection. Cells were stimulated 48 h after transfection with PMA/I for 3 h or with 90 mM K^+ for 3 min. For studies with nifedipine, Bay K8644 or AP-5, cells were pre-incubated for 3 min with the inhibitors before stimulation. The inhibitors were also present during stimulation. For experiments with FK506/CsA, ω-CgTx-GVIA and ω-CTx-MVIIC, the pre-incubation time was 20 min. For assays of spontaneous synaptic activity cells were transfected at 9 days *in vitro* and TTX or pharmacological inhibitors were added 16 h after transfection.

Subcellular fractionation and *in vitro* kinase assays

Nuclear extracts from newborn rat hippocampi were prepared using standard methods and analysed by western blot with a monoclonal antibody specific for GSK-3α and β (Santa Cruz). Kinase assays were done as described with minor modifications[15]. Samples were analysed by SDS–PAGE and autoradiography. The gel was stained with Coomassie blue to ensure equal loading of the fusion protein.

Plasmids and materials

Detailed description of the plasmids used in these studies can be found at http://crablab.stanford.edu/. Pharmacological agents were ionomycin (Calbiochem), phorbol-12-myristate-13-acetate (Calbiochem), tetrodotoxin (Calbiochem), Nifedipine (RBI), D(−)AP-5 (RBI), S(−)BayK 8644 (RBIU), ω-CgTx-GVIA (RBI), ω-CTx-MVIIC (RBI), FK506 (Fujisawa) and cyclosporin A (Sandoz).

Gel mobility shift assays

Nuclear extracts were prepared from newborn rat hippocampi and cerebellum and from NF-ATc4 transfected Jurkat T cells stimulated for 3 h with PMA and ionomycin and from PMA-stimulated JST-B cells as described[6] with minor modifications for the neuronal extracts. Binding reactions and electrophoretic mobility shift assays were carried out as described[6]. The oligonucleotide sequence of the ^{32}P-end-labelled oligonucleotide from the putative IP_3R1 NF-AT-binding site 2 from the 5′ flanking region of the IP_3R1 gene was 5′-TGACACCCGGGAAAGTTTGTGGAATGAATACGT-3′. The nucleotide sequence from the distal NF-AT binding site from the human IL-2 promoter (ARRE) was 5′-GATCG-GAGGAAAAACTGTTTCATACAGAAGGCGT-3′ and the AP-1 oligonucleotide sequence was 5′-GGGGTGACTAGGGG-3′.

Western blots for IP_3R1

One-week-old cultured hippocampal neurons were treated with TTX for three days. Stimulations were done as described for the transcriptional assays. Whole cell RIPA lysates were analysed by western blot with a polyclonal antibody specific for IP_3R1, a gift from I. Bezprozvanny.

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Two subsets of memory T lymphocytes with distinct homing potentials and effector functions

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Naive T lymphocytes travel to T-cell areas of secondary lymphoid organs in search of antigen presented by dendritic cells[1,2]. Once activated, they proliferate vigorously, generating effector cells that can migrate to B-cell areas or to inflamed tissues[3–6]. A fraction of primed T lymphocytes persists as circulating memory cells that can confer protection and give, upon secondary challenge, a qualitatively different and quantitatively enhanced response[7–9]. The nature of the cells that mediate the different facets of immunological memory remains unresolved. Here we show that expression of CCR7, a chemokine receptor that controls homing to secondary lymphoid organs, divides human memory T cells into two functionally distinct subsets. CCR7⁻ memory cells express receptors for migration to inflamed tissues and display