Online Methods

Subjects. C57BL/6J adult male mice (2 to 4 months of age) were housed individually on a 12 hr/12 hr light/dark schedule with lights on at 7 A.M. (ZT 0). For electrophysiological analysis of NMDA receptor function, mice were studied at 6 weeks of age. Food and water were available *ad libitum* throughout the experiment. Each animal was handled daily for 6 days prior to sleep deprivation. For electrophysiological, biochemical, and gene expression experiments, starting at approximately ZT 5, mice were sleep-deprived (SD) in their home cages for 5 hours by gentle handling[1]. Non-sleep-deprived mice (NSD) were left undisturbed in their home cages. Using EEG and EMG recordings, we found that this form of sleep deprivation resulted in ~95% wakefulness in SD mice, compared with ~35% wakefulness during the same time period in NSD mice (data not shown). All experiments were approved by the Institution of Animal Care and Use Committee of the University of Pennsylvania, or by the Animal Studies Committee at the University of Toronto, and were carried out in accordance with all National Institutes of Health guidelines.

Behavior. Foreground fear conditioning experiments were performed as previously described[2]. Mice were acclimated to the training/testing room during 6 days of handling. Starting at ZT 4-5, mice were placed into the conditioning chamber (Med Associates, St. Albans, VT) and received a 2 s, 1.5 mA scrambled footshock 2.5 min after placement into the chamber. Mice were removed from the chamber 30 seconds after the shock. For contextual fear conditioning experiments, animals were placed in a novel chamber (Med Associates, St. Albans, VT) for 3 minutes, and received a 2-second, 1.5 mA footshock after 2.5 minutes. Half of the mice were deprived of sleep as described above for 5 hours following training. Mice received two intra-

peritoneal (IP) injections of rolipram (1 mg/kg) or vehicle (2% DMSO in 0.9% saline) immediately and 2.5 hours after training. For trained context testing, mice received one 5 minute exposure to the same conditioned context in the absence of shock 24 h after conditioning. For altered context testing, mice received one 5 minute exposure to a novel context (another conditioning chamber with smooth flat floor, altered dimensions, and a novel odorant), 48 hours after conditioning. Conditioning was assayed by measuring freezing behavior, the complete absence of movement[3]. Freezing was scored during conditioning as well as testing. The behavior of each mouse was sampled at 5 s intervals, and the percentage of those intervals in which the mouse froze was calculated. Context-specific freezing was calculated by subtracting the freezing level in the altered context from the freezing level in the trained context for each animal.

Electrophysiology. Immediately following sleep deprivation, SD and time-matched NSD mice were sacrificed by cervical dislocation and their hippocampi were dissected rapidly in iced oxygenated ACSF. Field potential recordings were carried out as previously reported[2]. Briefly, 400μm thick transverse hippocampal slices were placed in an interface chamber[4] and continuously perfused with oxygenated ACSF while they equilibrated for at least 1.5 hours at 30° before starting electrophysiological recordings. A bipolar stimulating electrode (A-M systems, Inc., Sequim, WA; 0.002" diameter nichrome wire) placed in the stratum radiatum was used to elicit action potentials in the Schaffer collateral pathway. An ACSF-filled glass microelectrode (A-M systems, Inc. Sequim, WA; 1.5mm x 0.85mm) with a resistance between 0.5 and 3 MΩ placed in the stratum radiatum region of CA1 and was used to record the resulting field excitatory postsynaptic potentials (fEPSPs). Data were acquired using Clampex 8.2 (Axon Instruments, Inc. Union City, CA) and analyzed using Clampfit 8.2 (Axon Instruments, Inc.

Union City, CA). Peak fEPSP amplitude was required to be at least 3 mV, and stimulus intensity was set to produce 40% of the maximal response. Stimulations occurred every minute, and a 20-minute baseline period was recorded in each experiment, and recordings continued for at least 2 hours following LTP induction. Initial fEPSP slopes were normalized against the average of the 20 baseline traces.

1-train LTP was induced by a single 100 Hz, 1-second duration train of stimuli. This form LTP has been shown to be dependent on the NMDA receptor[5], calcium influx, and CaMKII activation, but is independent of PKA or translation[6,7]. 4-train LTP, an NMDA-receptor dependent form of LTP[8] consisted of 4 such trains applied with a 5-minute inter-train interval. The long-term maintenance of this form of LTP has an additional reliance on cAMP, PKA, transcription, and translation[6,9]. Massed 4-train LTP also used 4 such trains of stimuli, but with a 5-second inter-train interval. This form of L-LTP, despite being dependent on the NMDA receptor and translation, does not require PKA[8]. Theta-burst stimulation (TBS) consisted of 40-ms duration, 100 Hz bursts delivered at 5 Hz for 3 seconds (15 bursts of 4 pulses per burst, for a total of 60 pulses). The induction of this form of LTP depends on the NMDA receptor and the maintenance of this form of LTP depends on cAMP[10]. Chemical LTP was induced by treatment of slices for 15 minutes with either 50 μM molecular grade forskolin, an adenylate cyclase activator[11] (FSK; Sigma-Aldrich, St. Louis, MO) in 0.1 % ethanol, or a combination of 50 μM forskolin and the broad-spectrum PDE inhibitor 30 μM 3-isobutyl-1-methylxanthine[12] (IBMX; Tocris Cookson, Inc. Ellisville, MO); IBMX was dissolved in water. The cAMP-specific PDE4 inhibitor rolipram[13] (ROL: Sigma-Aldrich, St. Louis, MO) was applied at 0.1 μM in 0.1% DMSO (Sigma-Aldrich) for 60 minutes, beginning 30 minutes prior to tetanization. In all LTP figures, representative sample sweeps are shown for each LTP experiment. The red sweeps

represent the average of the first 5 baseline sweeps, and the black sweeps represent the last 5 sweeps in the recording. All scale bars are 5 mV/5ms.

Input-output characteristics were examined by recording fEPSPs in CA1 resulting from stimuli of increasing intensity. Initial fEPSP slopes were plotted against the corresponding presynaptic fiber volley amplitudes, and the resulting plots were fit with linear regressions. The slope of the maximum elicited fEPSP in each slice was also recorded. Paired-pulse facilitation, a short-term form of synaptic plasticity, was measured in slices from sleep-deprived and control mice. Pairs of stimuli were delivered with varying delays (300, 200, 100, 50, 25 ms) between the two stimuli, and the initial fEPSP slope from the second stimulus was plotted relative to the slope from the first stimulus.

For whole-cell patch clamp recordings in slices from SD and NSD mice, coronal brain slices (350 μm) containing the hippocampus were prepared as previously described[14]. Slices were kept in a submerged recovery chamber at room temperature for at least 1 hour before electrophysiological experiments, in oxygenated (95 % O2 and 5 % CO2) artificial cerebrospinal fluid (ACSF) containing the following (in mM): 124 NaCl, 2.5 KCl, 2 CaCl2, 2 MgSO4, 25 NaHCO3, 1 NaH2PO4, and 10 glucose. Whole-cell patch clamp recording experiments were performed in a recording chamber on the stage of an Olympus BX51WI microscope (Tokyo, Japan) with infrared DIC optics for visualization of whole-cell patch clamp recordings. The recording pipettes (3-5 MΩ) were filled with solution containing (in mM): 102 CsMeSO3, 3.7 NaCl, 5 QX-314 chloride, 5 TEA-Cl, 10 BAPTA, 0.2 EGTA, 20 HEPES, 0.3 Mg-ATP, and 0.3 Na3-GTP (adjusted to pH 7.2 with CsOH). Excitatory postsynaptic currents (EPSCs) were recorded from hippocampal CA1 pyramidal neurons with an Axon 200B amplifier (Molecular devices, CA) and the stimulations were delivered by a bipolar tungsten stimulating electrode

placed in striatum radium. Data were collected and analyzed using pClamp 9.2 software. Picrotoxin (100 μM) was always present to block $GABA_A$ receptor-mediated inhibitory synaptic currents. AMPA receptor-mediated EPSCs were induced by repetitive stimulations at 0.05 Hz, and neurons were voltage clamped at -70 mV (liquid junction potential corrected). The NMDA receptor-mediated EPSCs were recorded in the presence of CNQX (20 μM) and evoked at 0.05 Hz. Neurons were voltage-clamped at 0 mV for the experiments examining the input-output relationship of NMDA receptor-mediated EPSCs. Data were discarded if access resistance changed by more than 15% during experiments.

Biochemistry. For cAMP assays, hippocampal slices were prepared as for electrophysiology, and were treated for 15 minutes with FSK (50 μM), FSK plus IBMX (30 μM), or vehicle. At 10 minutes after drug treatment, slices were removed from the rig and the CA1 region was dissected and flash-frozen in a dry ice/ethanol slurry. Samples were stored at -80°C until enough samples had been accumulated to run the assay. For the assay itself, a cAMP [I^{125}] radioimmunoassay kit was used (PerkinElmer, Shelton, CT). Tissue was homogenized on ice in 6% TCA, a set amount (~30,000 cpm) of tritiated cAMP (adenosine 3`, 5`-cyclic phosphate, ammonium salt, [2,8-^{3}H]; PerkinElmer) was added to each sample, and extracts were centrifuged at 2500g at 4°C for 15 minutes. The pellet was frozen and used later in Bradford assays to determine the total protein concentration in each sample. After extracting the supernatants 4 times with water-saturated ether, samples were evaporated in a speed-vac and then resuspended in assay buffer. Recovery was assessed by measuring the amount of [^{3}H] cAMP in each sample using a scintillation counter. Assay was run according to kit instructions for non-acetylated procedure, and reaction was run at 4°C overnight. I^{125} was measured using a gamma scintillation counter. Based on

Bradford assays, recovery assessment, and standard curves, cAMP levels (pmol/mg protein) were calculated for each sample. Because of inter-assay variability, raw data was normalized for all groups relative to the NSD control average for each assay. Data is therefore presented as % NSD control cAMP level.

For PDE activity assays, hippocampi were dissected from SD and NSD mice and immediately frozen in a dry ice/ethanol slurry. The cyclic AMP-specific PDE activity assay was adapted from the radioassay procedure of Marchmont and Houslay[15], as described previously[16]. In this two-step procedure the samples to be assayed are (in the first step) incubated with 1 μM 8-[^{3}H]-labeled cAMP substrate for 10 minutes at 30°C, in the absence or presence of rolipram (10 μm) to determine the rolipram-inhibited PDE4 activity. In the second step, the [^{3}H]-labeled cAMP product of cAMP hydrolysis, 5'AMP, was dephosphorylated to adenosine by incubation with 0.2 mg/ml snake venom. The negatively charged unhydrolyzed cAMP was then separated from the uncharged adenosine by incubation with Dowex ion exchange resin (Dowex-1-chloride); this removes the charged nucleotides but not the uncharged nucleosides. The amount of unbound [^{3}H]-adenosine in the supernatant was determined by scintillation counting in order to calculate the rate of cAMP hydrolysis. PDE4-specific activity was calculated by subtracting the activity found in the presence of rolipram from the activity in the absence of rolipram.

For PDE4 Western blots, hippocampi were flash-frozen on a dry ice/ethanol slurry and were stored at -80°C. 154μg of total protein was resolved using NuPAGE® 4-12% Bis-Tris gel and NuPAGE® MOPS SDS running buffer (Invitrogen, Carslbad, CA) for 1 hour at 190V. The separated proteins were transferred to nitrocellulose membrane (Protran®, Whatman GmbH, Dassel, Germany) for 1 hour at 25V using NuPAGE® Transfer buffer. The membranes were blocked in 5% marvel/TBS-T for at least 1 hour with gentle shaking. Antibodies specific for

PDE4A, PDE4B, PDE4D, PDE4A5, PDE4A10, PDE4A11 (1:1000 for each)[17,18] or α-tubulin (1:5000, mouse) (Sigma-Aldrich Co., St. Louis, MO) were added in 1% marvel/TBS-T and incubated at 4°C overnight. The membranes were washed 3 times for 10 minutes in TBS-T. Peroxidase-conjugated anti-rabbit (Sigma-Aldrich Co.) or anti-mouse (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK) were added 1:2000 in 1% marvel/TBST and incubated for 1 hour at room temperature. Membranes were washed as before, then incubated in Pierce® ECL Western Blotting Substrate (Thermo Scientific, Rockford, IL) for 30 seconds and the signal was detected using film (Kodak, Carestream Health, Inc., Rochester, NY). Densitometry was performed using Quantity One software (BioRad Laboratoriese, Hercules, CA).

For phospho-CREB immunohistochemistry, SD and NSD mice were anesthetized with isoflurane and perfused with PBS followed by 4% paraformaldehyde in PBS. Fixed brains were dissected and then cryoprotected in 30% sucrose in PBS overnight at 4^{O}C. Brains were mounted on cryostat chucks using OCT. Coronal sections were cut at a thickness of 25 μm and stored in PBS with 0.1% sodium azide. After rinsing in PBS (3x), incubation in 0.3% H_2O_2 (30 min), and rinsing again in PBS (3x), sections were preincubated in 5% normal goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA), in PBS for 20 min at room temperature. After preincubation, sections were incubated with rabbit-anti-pCREB IgG (1:1500; Upstate Cell Signaling Solutions, Charlottesville, VA) in 1% normal goat serum, 0.3% triton X-100 and 0.1% azide in PBS at room temperature for 24 hours. Sections were then rinsed in PBS (3x) and incubated for 3 hours with biotinylated goat-anti-rabbit IgG (1:500; Jackson immunoResearch Laboratories) in PBS containing 0.3% triton X-100 and 1% normal goat serum at 5°C. After rinsing with PBS (3x), sections were incubated with the avidin-biotin-horseradish peroxidase

complex (1:500 ABC kit, Vector laboratories Burlingame, CA) in PBS containing 0.3% triton X-100 for 2 hours at room temperature. Finally after rinsing in PBS for 6 hours, sections were processed with diaminobenzidine (0.02% in Tris-HCl pH 7.6) with 100 μl 0.1% H_2O_2 as a reaction initiator. After 7 minutes, the reaction was terminated by washing the sections with PBS. Sections were mounted with gelatin (0.7%) and dried for 24 hours. The mounted sections were then dehydrated using ethanol and xylene, coverslipped with DPX (Merck), and dried for 24 hours. Relative optical densities (OD) of pCREB immunoreactivity were quantified in subregions of the dorsal hippocampus (3 sections per animal, averaged for both hemispheres) and in the amygdala (3-8 amygdala) using ImageJ image analysis software. The OD is expressed in arbitrary units corresponding to grey levels. The OD values for each region were normalized to the background labeling within the same region to normalize for variability in staining between sections.

For corticosterone assays, animals were sacrificed by cervical dislocation in control mice or mice that had been sleep-deprived for 5 hours, and trunk blood was collected. After 30-60 minutes at room temperature, samples were centrifuged at 5,000 rpm for 10 minutes to isolate the supernatant (plasma), which was then stored at -80°C until enough samples had been accumulated to run the assay. Corticosterone concentrations were measured using a commercial ^{125}I radioimmunoassay kit (MP Biomedicals, Orangeburg, NY) according to instructions. Samples were run in duplicate. The minimum detection limit of the assay was 7.7 ng/mL and the inter-replicate variability was 6.0%.

Gene Expression Analysis. For quantitative real-time RT-PCR experiments, mice were handled and sleep deprived as described above. Hippocampal dissections were performed immediately

following the deprivation, and alternated between SD and NSD animals. RNA preparation and real-time RT-PCR was performed as previously described[2]. Briefly, hippocampi were flash-frozen in 500 μL RNAlater (Ambion, Austin, TX) on dry ice and stored at -80°C. Aerosol Barrier ART tips, DEPC-treated sterile water (Ambion), and autoclaved 1.5 mL microcentrifuge tubes were used for all subsequent steps. Thawed hippocampi were homogenized on ice in 1 mL Trizol (Invitrogen, Carlsbad, CA) using a dounce homogenizer. Phenol-chloroform (300 µL) addition was followed by vigorous mixing and room temperature incubation for 3 minutes. Samples were transferred to phaselock gel tubes (Eppendorf, Westbury NY) and were centrifuged at 4°C and full speed for 15 minutes to extract RNA. The aqueous phase was transferred to new tubes, where 2 volumes of ethanol, 1/10 volume 3M NaOAc, and 1 µl glycogen (10 mg/mL) were added. After gentle mixing, the samples were incubated at -20°C for ten minutes. Centrifugation at 4°C and full speed was followed by aspiration of the supernatant. Pellets were washed with 300 µl 70% ethanol, and then centrifuged at 4°C and full speed for 5 minutes. The supernatant was aspirated and samples were air-dried for approximately 10 minutes. After resuspension in 100 µl water, RNA was purified using the RNeasy system (Qiagen, Valencia CA) according to the manufacturer's protocol. Residual DNA was removed by treatment with *DNA-free* (Ambion, Austin, TX). RNA was then re-precipitated with 1 μL glycogen, 50 μL NH4Ac, and 250 μL ethanol. Pellet was then rinsed twice with 300 μL 80% ethanol, and resuspended in 24 μL RNAse-free water. RNA concentration and purity was quantified by NanoDrop spectrophotometry (Thermo Fisher Scientific, Wilmington, DE).

Generation of cDNA was carried out by the RETROscript kit (Ambion). Each reaction was performed with 1 µg RNA in a total volume of 20 µl composed as follows: 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 5 mM DTT, 500 µM each dNTP, 5 µM random decamers, 10

units RNase inhibitor, and 100 units MMLV-RT. As controls, additional reaction mixtures were generated that lacked either reverse transcriptase or template mRNA. Subsequent reactions were allowed to proceed at 44°C for one hour, followed by heat inactivation at 100°C for ten minutes.

For quantitative real-time RT-PCR, reactions were prepared in 96-well optical reaction plates (ABI, Foster City, CA) with optical adhesive covers (ABI). Each well contained 11.4 μl cDNA, 1 μl of 5 μM primer mix solution, and 12.4 μl Power SYBR Green PCR Master Mix (ABI). Three technical replicates were used. Reactions were carried out in the ABI Prism 7000 with an initial activation at 50°C for 2 minutes followed by incubation at 95°C for 15 minutes and 40 subsequent cycles of 95°C for 15 sec, 56°C for 30 sec, and 72°C for 30 sec. Primer sequences were as follows: *PDE4A*: forward – CACCTTCCTGCTGCTTCTCT, reverse – CTGCTGGAAGGGTCTCTGTC. *PDE4B*: forward – CAGATCAGGGAACCAGGTGT, reverse – AGCGTGAGATGCTTGTGTTG. *PDE4D*: forward – AGATGAGTCGGTCTGGCAAC, reverse – CGGCCTTTTCTTCTTCTCCT. Data was normalized to *Actg1*, *Hprt* and *Tuba4a* prior to calculation of differences. Housekeeping primers were the same as described previously[2]. Relative quantification of gene expression was performed according to ABI's User Bulletin #2. Fold change was calculated from the delta Ct values with corrections for standard curve data from each gene and housekeeping gene expression levels for each sample based on the relative standard curve method described in the Applied Biosystems manual. For each sample, the delta Ct was calculated against the mean for that gene's sample set. Next, each of these delta Ct values was corrected with the slope of the standard curve for the relevant primer set to account for any variation in primer amplification efficiency. The efficiency-corrected delta Ct value was normalized to the similarly corrected delta Ct from housekeeping genes for each sample to account for variability in mRNA input. The

difference between corrected delta Ct for each sample was then grouped according to group (Sleep-deprived or non-sleep-deprived) and the average delta Ct calculated. Because Ct values are on a logarithmic scale, fold change is equal to two raised to the difference between experimental and control delta Ct values. Because corrections were made for primer efficiency, we have presented the data as fold change. The data presented is the calculated mean for the biological replicates with n being equal to the number of biological replicates (*i.e.*, the number of mice examined).

Statistics. For LTP experiments, the maintenance of LTP was analyzed using repeated measures one-way ANOVAs with Tukey post-hoc tests, with group and time as factors, and the percent baseline fEPSP slope during the last 20 minutes of the recording as the variable. For experiments using rolipram treatment, repeated measures two-way ANOVAs were used, with group and treatment as factors. Analysis of LTP induction was carried out using t-tests, except in the case of experiments using rolipram, in which 2-way ANOVAs were used, with group and treatment as factors. To evaluate differences in input-output characteristics, a t-test was performed comparing the average linear regression slopes for sleep-deprived and control mice. For paired-pulse facilitation, a repeated measures two-way ANOVA with Tukey post-hoc tests was used with group and inter-stimulus interval as factors and the ratio of the initial fEPSP slopes elicited by the two stimuli as the variable. For whole-cell patch clamp recordings, NMDA receptor I-V curves were analyzed using a 2-way ANOVA with voltage and sleep deprivation as factors. NMDA receptor input-output curves were compared using a 2-way ANOVA with stimulation intensity and sleep deprivation as factors. NMDAR/AMPAR ratios were compared between SD and NSD animals using a t-test. For cAMP assays, a non-parametric Kruskal-Wallis test was

used because data across treatment groups was non-normal. An overall effect of group was found ($H(5, N=108) = 42.04$, $p < 0.0001$), and p-values reported in the text are from *post-hoc* Mann-Whitney U tests used to make the following planned comparisons: NSD control vs. SD control, NSD + FSK vs. SD + FSK, and NSD + FSK/IBMX vs. SD + FSK/IBMX. For PDE activity assays and PDE4 Western blots, t-tests were used to compare values between SD and NSD animals, using 1-tailed p-values because of our prediction that PDE levels and activity would be increased by sleep deprivation. For pCREB immunohistochemistry, t-tests were used to compare optical densities in each hippocampal region, using 1-tailed p-values because of our initial prediction that CREB phosphorylation would be reduced by sleep deprivation. For quantitative real-time RT-PCR experiments, t-tests were used to compare fold change values for each gene between sleep-deprived and control mice, and 1-tailed p-values are reported because of our initial prediction that PDE expression would be increased by sleep deprivation. 2-tailed p-values are reported for RT-PCR experiments examining the effects of recovery sleep. For fear conditioning experiments, a two-way repeated measures ANOVA with Tukey *post hoc* tests was used, with group (SD vs. NSD) and drug treatment (ROL vs. VEH) as factors, and context (trained, altered) as the repeated measure, and percent freezing as the variable. Effects on context-specific freezing were measured using a two-way ANOVA with Tukey *post hoc* tests, with group and drug treatment as factors, and the difference in percent freezing between the trained and altered contexts as the variable. All statistics were carried out using Statistica 7 (Statsoft, Inc., Tulsa, OK). In all figures in the main text, * represents a comparison with $p < 0.05$, and all error bars indicate +/- SEM.

Online Methods References

1 Ledoux, L., Sastre, J. P., Buda, C., Luppi, P. H. & Jouvet, M. Alterations in c-fos expression after different experimental procedures of sleep deprivation in the cat. *Brain research* **735**, 108-118 (1996).

2 Vecsey, C. G. *et al*. Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. *J Neurosci* **27**, 6128-6140 (2007).

3 Fanselow, M. S. Conditioned and unconditional components of post-shock freezing. *Pavlov J Biol Sci* **15**, 177-182 (1980).

4 Matthies, H. *et al*. Design of a multiple slice interface chamber and application for resolving the temporal pattern of CREB phosphorylation in hippocampal long-term potentiation. *J Neurosci Methods* **78**, 173-179 (1997).

5 Malleret, G. *et al*. Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. *Cell* **104**, 675-686 (2001).

6 Abel, T. *et al*. Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell* **88**, 615-626 (1997).

7 Huang, Y. Y. & Kandel, E. R. Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. *Learn Mem* **1**, 74-82 (1994).

8 Woo, N. H., Duffy, S. N., Abel, T. & Nguyen, P. V. Temporal spacing of synaptic stimulation critically modulates the dependence of LTP on cyclic AMP-dependent protein kinase. *Hippocampus* **13**, 293-300 (2003).

9 Nguyen, P. V., Abel, T. & Kandel, E. R. Requirement of a critical period of transcription for induction of a late phase of LTP. *Science (New York, N.Y* **265**, 1104-1107 (1994).

10 Nguyen, P. V. & Kandel, E. R. Brief theta-burst stimulation induces a transcription-dependent late phase of LTP requiring cAMP in area CA1 of the mouse hippocampus. *Learn Mem* **4**, 230-243 (1997).

11 Daly, J. W., Padgett, W. & Seamon, K. B. Activation of cyclic AMP-generating systems in brain membranes and slices by the diterpene forskolin: augmentation of receptor-mediated responses. *J Neurochem* **38**, 532-544 (1982).

12 Beavo, J. A. *et al*. Effects of phosphodiesterase inhibitors on cyclic AMP levels and on lipolysis. *Ann N Y Acad Sci* **185**, 129-136 (1971).

13 Conti, M. & Beavo, J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem* **76**, 481-511 (2007).

14 Wu, L. J. *et al*. Neurabin contributes to hippocampal long-term potentiation and contextual fear memory. *PLoS ONE* **3**, e1407 (2008).

15 Marchmont, R. J. & Houslay, M. D. Insulin trigger, cyclic AMP-dependent activation and phosphorylation of a plasma membrane cyclic AMP phosphodiesterase. *Nature* **286**, 904-906 (1980).

16 Lobban, M., Shakur, Y., Beattie, J. & Houslay, M. D. Identification of two splice variant forms of type-IVB cyclic AMP phosphodiesterase, DPD (rPDE-IVB1) and PDE-4 (rPDE-IVB2) in brain: selective localization in membrane and cytosolic compartments and differential expression in various brain regions. *Biochem J* **304 (Pt 2)**, 399-406 (1994).

17 Huston, E. *et al*. The cAMP-specific phosphodiesterase PDE4A5 is cleaved downstream of its SH3 interaction domain by caspase-3. Consequences for altered intracellular distribution. *J Biol Chem* **275**, 28063-28074 (2000).

18 Lynch, M. J. *et al*. RNA silencing identifies PDE4D5 as the functionally relevant cAMP phosphodiesterase interacting with beta arrestin to control the protein kinase A/AKAP79-mediated switching of the beta2-adrenergic receptor to activation of ERK in HEK293B2 cells. *J Biol Chem* **280**, 33178-33189 (2005).