Methods:

**Recombinant expression of PKMζ and PKMζ-K281W.** Spodoptera frugiperda (Sf9) cells were grown in SF-900 II Serum Free Medium (Gibco, Rockville, MD) containing 5 µg/ml gentamicin. To express PKMζ, 10⁹ cells of a log-phase Sf9 culture were spun and infected with the baculovirus-PKCζ virus stock (generous gift from S. Stabel, Max Planck Institute, Cologne, Germany). The cells were seeded at a density of 10⁶ cells/ml. After 3 days, the cells were spun, washed with phosphate-buffered saline, and sonicated in 65 ml of homogenization buffer (20 mM Tris-HCl [pH 7.5], 2 mM EDTA, 10 mM EGTA, 0.3% β-mercaptoethanol, 20 µg/ml aprotinin, 5 mM benzamidine, 0.1 mM leupeptin, 70 µg/ml 4-(2-aminoethyl)benzenesulfonylfluoride). A 2-step purification of PKMζ from PKCζ, employing DEAE Fast Flow Sepharose and Superdex 75 columns (preparation grade, Pharmacia, Piscataway, NJ), was performed to purify PKMζ to near homogeneity, as analyzed by silver stain (Supplementary Fig. 1) and immunoblot with carboxy-terminal ζ-antiserum⁷ (not shown).

PKMζ-K281W was expressed using the MaxBac 2.0 baculovirus/Sf9 system (Invitrogen, Carlsbad, CA). An insert containing amino acids 158-592 of PKCζ-K281W (generous gift from A. Romanelli, A. Toker and J. Blenis, Harvard Medical School, Boston, MA) was subcloned into the baculovirus transfer vector pBlueBacHis2B between the EcoRI and SalI sites. The construct was sequenced for verification and then cotransfected into Sf9 cells with linearized baculovirus genome. Recombinant protein was expressed in Sf9 cells, purified using a Ni²⁺ column (Invitrogen, Xpress™ Purification Kit, Carlsbad, CA), and analyzed by silver stain (Supplementary Fig. 9a) and immunoblot with carboxy-terminal ζ-antiserum⁷ (not shown). Final concentrations of recombinantly expressed proteins were determined by silver staining within the linear range for each protein, with bovine serum albumin as standard.

**Kinase activity.** Activity of the PKMζ used in whole-cell recordings was assayed on the same day as electrophysiological experiments. The reaction mixture (50 µl final volume) contained: 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol, and 25 µM PKCζ substrate (Peninsula Laboratories, San Carlos, CA). The reaction, begun with the addition of 50 µM ATP (4 µCi [γ-³²P]/assay), was for 10 min at 30°C, which is in the linear range for time and enzyme concentration (data not shown). The reaction was stopped by addition of 25 µl of 100 mM cold ATP and 100 mM EDTA, and 40 µl of the assay was spotted onto phosphocellulose paper and counted by liquid scintillation. PKM activity was measured as the difference between counts incorporated in the presence and absence of enzyme.

PKC activity was measured as the difference between counts incorporated in the
presence and absence of activators, 10 µg/ml phosphatidylserine and 400 nM phorbol 12-myristate 13-acetate (and 0.2 mM CaCl$_2$ for conventional PKC$\alpha$). Chelerythrine competes for protein substrate binding to PKC’s catalytic domain (Herbert, J. M., Augereau, J. M., Gleye, J. & Maffrand, J. P. Biochem. Biophys. Res. Comm. 172, 993-999 [1990]). Therefore, myelin basic protein (400 nM, Sigma, St. Louis, MO) was chosen as the PKC substrate because it was found to have a similar Km (~175 nM) for the PKC isoforms tested. CaMKII activity was measured as the difference between counts incorporated in the presence and absence of the activators, 6 µM calmodulin and 0.5 mM CaCl$_2$, using syntide-2 as substrate (20 µM, twice its Km, Hashimoto, Y. & Soderling, T. R. Arch. Biochem. Biophys. 252, 418-425 [1987]). The myristoylated $\zeta$-pseudosubstrate peptide (myr-SIYRRGARRWRKL, BioSource International, Camarillo, CA) inhibited PKC$\alpha$ less than PKM$\zeta$, but the IC50 for PKC$\alpha$ was variable due to interactions between phosphatidylserine micelles and the myristoylated peptide. Unless otherwise stated, kinases were from Calbiochem (San Diego, CA).

**Whole-cell recordings in hippocampal slices.** Experimental animals were used in accordance with the State University of New York, Downstate Medical Center Institutional Animal Use Committee. Hippocampal slices were prepared from Sprague-Dawley rats (P18-P30), as previously described (Ling, D. S. F. & Benardo, L. S. J. Neurophysiol. 82, 1793-1807 [1999]). Rats were anesthetized with ketamine and xylazine, decapitated, and their brains quickly removed and placed in chilled (0-5°C) physiological saline (see below). Hippocampi were isolated by free hand dissection and cut into transverse slices (300-400 µM) using a Vibratome tissue sectioner (St. Louis, MO). Slices were then transferred to an antechamber and incubated in oxygenated saline at 35.5°C for a minimum of 1 hr. Following incubation, single slices were transferred to a 2.0 ml recording chamber placed on the stage of an upright microscope (Zeiss Axiomkop II) and perfused with oxygenated saline at a rate of approximately 5 ml/min at 34-35°C. Physiological saline consisted of (in mM): 124 NaCl, 5 KCl, 26 NaHCO$_3$, 1.6 MgCl$_2$, 4 CaCl$_2$, 10 glucose (pH between 7.35 and 7.4), and was continuously bubbled with a mixture of 95% O$_2$-5% CO$_2$.

Patch electrodes were pulled (two-stage) from 1.5 mm O.D. borosilicate glass (World Precision Instruments, Sarasota, FL) on a Narishige PP-83 vertical puller (Tokyo, Japan). Recording pipettes had tip resistances of 2-5 MΩ and contained (in mM): 130 Cs-gluconate, 10 NaCl, 0.2 EGTA, 10 HEPES, 2 Na-ATP, 0.5 Na-GTP, pH adjusted to 7.25 with CsOH. Cesium was used to block potassium currents, including slow GABA$_B$ inhibitory postsynaptic currents.

Whole-cell recordings were obtained from CA1 pyramidal cells visualized in slices (Ling, D. S. F. & Benardo, L. S. J. Neurophysiol. 82, 1793-1807 [1999]). Signals were
recorded under voltage-clamp with a Warner PC-501 amplifier (Warner Instruments, Hamden, CT). Brief voltage steps (-10 mV, 10 ms) from cell holding potential were applied to monitor cell access resistance, capacitance and input resistance, in order to assess the stability of passive membrane properties during the recordings. Cells were accepted for study only if resting input resistances of >100 MΩ and access resistances <20 MΩ were observed. If cell access resistance increased significantly during the course of the recording (>20%), the data were discarded. Data signals were digitized at 94 kHz via a 14-bit PCM interface (VR-10B Digital Data Recorder, Instrutech Corp., Elmont, NY) and stored on VHS tape for later analysis with pCLAMP software (Axon Instruments, Foster City, CA) on an IBM-compatible Pentium-II microcomputer. Simultaneous field recordings were obtained by an extracellular electrode, containing 2 M NaCl, placed in CA1 stratum radiatum.

Synaptic events were evoked by extracellular stimulation with bipolar, coated tungsten electrodes placed in stratum radiatum lateral to the recording electrode. Cathodal shocks (2-10 V; 200 µs duration) were delivered through a digitally controlled stimulus isolation unit (World Precision Instruments, Sarasota, FL) at a low frequency (0.1 Hz). LTP was produced by four 100 Hz-1 sec trains at test stimulation intensity, separated by 20 sec⁸, which were paired with continuous depolarization to −40 mV of the whole-cell recorded neuron. For field recordings in Fig. 3, four tetanic trains of stimuli at 75% of the maximal EPSP response were delivered 5 min apart in an interface chamber as previously described⁷. For two pathway experiments, stimulating electrodes were placed on either side of the recording electrode in stratum radiatum, and separation of the synaptic pathways demonstrated by the absence of paired–pulse facilitation between the two responses.

Spontaneous EPSCs were detected and measured using Mini Analysis 4.0 software (Synaptosoft Inc., Leonia, NJ), which identifies spontaneous currents on the basis of several criteria, including threshold amplitude and the area under each event (Ling, D. S. F. & Benardo, L. S. J. Neurophysiol. 82, 1793-1807 [1999]). As a routine check, we visually inspected all sEPSCs detected by the software and rejected any events that did not exhibit the general shape expected for synaptic events. Background noise was measured from quiescent sections of records (i.e., devoid of spontaneous events). Peak-to-peak noise levels ranged from 2 to 8 pA. Unless otherwise noted, drugs were delivered in the bath.

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