PKMζ-PKCι/λ double-knockout reveals the atypical PKCs are crucial for hippocampal late-LTP and spatial long-term memory

Authors

Panayiotis Tsokas[1,2,3], Changchi Hsieh[1], Alejandro Grau-Perales[4], Leo Kwok[4], Laura Melissa Rodriguez-Valencia[1,2], David A. Cano[5], Kim D. Allen[1,3,6], Hannah J. H. Smith[1,7], Sabina Kubayeva[5], Benson J. Wei[1], Samuel Sabzanov[5], Rafael E. Flores-Obando[1], Sourav Ghosh[8], Peter John Bergold[1,9], James E. Cottrell[2], André Antonio Fenton[1,4,10]*, Todd Charlton Sacktor[1,2,9]*

Affiliations

[1]Department of Physiology and Pharmacology, The Robert F. Furchgott Center for Neural and Behavioral Science, State University of New York Downstate Health Sciences University; Brooklyn, New York 11203, USA.

[2]Department of Anesthesiology, State University of New York Downstate Health Sciences University; Brooklyn, New York 11203, USA.

[3]Department of Pathology, State University of New York Downstate Health Sciences University; Brooklyn, New York 11203, USA.

[4]Center for Neural Science, New York University; New York, New York 10003, USA.

[5]College of Medicine, State University of New York Downstate Health Sciences University; Brooklyn, New York 11203, USA

[6]Department of Biology, City University of New York- Medgar Evers College, Brooklyn, New York 11225, USA

[7]Currently, Department of Neuroscience and Cell Biology, Rutgers University; New Brunswick, New Jersey 08901, USA.

[8]Departments of Neurology and Pharmacology, Yale University; New Haven, Connecticut 06520, USA

[9]Department of Neurology, State University of New York Downstate Health Sciences University; Brooklyn, New York 11203, USA.

[10]Neuroscience Institute at NYU Langone Medical Center; New York, New York 10016, USA.

*Corresponding authors. Emails: tsacktor@downstate.edu, afenton@nyu.edu

Abstract

PKMζ, a persistently active, atypical PKC (aPKC) isoform, maintains enduring long-term potentiation (LTP) and memory in wild-type (WT) mice; however, hippocampal LTP and memory in PKMζ-knockout (ζ-KO) mice are maintained by compensation. Although ζ-KO eliminates neocortical LTP, hippocampal LTP may be sustained by another aPKC, ι/λ (PKCι). PKCι is critical for the generation of an early phase of LTP and short-term memory, and its genetic deletion is compensated by PKMζ. Using a double knockout strategy, deleting both PKCι and PKMζ (ι/ζ-dKO) in hippocampus, we report that ι/ζ-dKO eliminates enduring LTP and spatial long-term memory but shows an early-phase, short-lasting LTP and short-term memory. ZIP, an inhibitor of both PKMζ and PKCι that disrupts LTP in WT and PKMζ-null hippocampus, does not affect LTP in ι/ζ-dKO hippocampus, suggesting ZIP disrupts LTP by targeting PKMζ or PKCι. Thus, the double-KO reveals that additional compensatory short-term processes can be induced without aPKCs, whereas additional long-term processes cannot. We conclude that PKMζ, under normal conditions, and PKCι as an essential back-up are crucial for the persistence of hippocampal LTP and spatial memory.

Introduction

The search for molecules that maintain long-term potentiation (LTP) and memory revealed that under normal circumstances PKMζ, an atypical PKC (aPKC) isoform, plays a critical role in maintaining the enduring late-phase of LTP and long-term memory (*Sacktor et al., 1993; Pastalkova et al., 2006; Wang et al., 2016; Tsokas et al., 2024*). Experiments genetically deleting PKMζ have found that the loss of PKMζ can be compensated in hippocampus so that LTP appears normal (*Tsokas et al., 2016; Tsokas et al., 2024*), but cannot be compensated in neocortex where LTP is eliminated (*Kniffin et al., 2025*). Analysis of the PKC gene family of 9 conventional, novel, and atypical isoforms showed that the most closely related gene, the other aPKC – PKCι/λ (hereafter referred to as PKCι), compensates for the loss of PKMζ in hippocampus. PKCι has been reported critical to the initial generation of the early phase of LTP and short-term memory (*Ren et al., 2013; Tsokas et al., 2016; Wang et al., 2016; Sheng et al., 2017*). Furthermore, genetic deletion of PKCι results in an early-LTP and short-term memory that is compensated by PKMζ (*Sheng et al., 2017*).

These results raise two questions. One, does PKCι alone substitute for PKMζ to support the late phase of LTP and long-term memory. Two, does PKMζ alone substitute for PKCι to mediate the early phase of LTP and short-term memory? To answer these questions, we employed a double knockout strategy, deleting both PKCι and PKMζ (ι/ζ-dKO). Note, if only PKCι can substitute for PKMζ then the double-knockout animals will not exhibit late-LTP and long-term memory. In addition, if only PKMζ can substitute for PKCι then double-knockout animals will lack early-LTP and short-term memory.

We report that mice with double-knockout of both PKCι and PKMζ in hippocampus do not display late-LTP and long-term memory but show early-phase LTP and short-term memory. We conclude that PKCι exclusively substitutes for PKMζ to maintain the late-phase LTP and long-term memory, and PKCι may not be the only molecule that supports transient forms of LTP and memory.

Results

We investigated whether the genetic compensation that occurs in the hippocampus of mice with constitutive PKMζ-KO (PKMζ-null mice) also occurs in a conditional PKMζ-KO (PKMζ-cKO). PKMζ-null mice increase basal levels of PKCι and the conventional PKCβI (*Tsokas et al., 2016*). Tamoxifen injections conditionally ablated PKMζ in adult CaMKIIα promoter-driven-CreER^{T2}Prkcz$^{fl/fl}$ mice. One week later, expression of PKMζ decreased and there was a compensatory increase in PKCι, as well as PKCι phosphorylated on its activation-loop (Figure 1A, Figure 1 — figure supplement 1A, Tables 1A, 2A). Similar increases are seen in PKMζ-null mice (*Tsokas et al., 2016*). The expression of all four conventional PKCs (α, βI, βII, γ) increased, as did phosphorylation of the conventional PKC activation-loop (Figure 1B, Figure 1 — figure supplement 1A, Tables 1B, 2A). The four novel PKC isoforms (δ, ε, η, θ) and the activation-loop phosphorylation of PKCε do not change (Figure 1B, Figure 1 — figure supplement 1A, Tables 1B, 2A). Levels of CaMKIIα, critical for LTP induction (*Bayer and Giese, 2024*), and T286-autophosphorylation of CaMKIIα, which induces Ca^{2+}-independent autonomous kinase activity (*Miller and Kennedy, 1986*), also do not change (Figure 1 — figure supplement 1B).

These data show that the hippocampus of conditional and constitutive PKMζ-KO mice express similar compensatory increases in PKCι as well as other PKCs.

During long-term spatial memory storage, PKMζ increases for at least one month in CA1 *stratum (st.) radiatum* of wild-type (WT) mice (*Hsieh et al., 2021; Han et al., 2025*). We tested if *st. radiatum* of PKMζ-cKO mice have similar compensatory increases of PKCι. PKMζ was ablated in CaMKIIα-promoter-driven-CreER^{T2}Prkcz$^{fl/fl}$ mice. Three weeks later, the PKMζ-ablated mice were trained on active place avoidance (Figure 1 — figure supplement 2). One week after training, PKMζ in CA1 *st. radiatum* of the PKMζ-cKO decreased to ~25% and PKCι increased to ~400%, compared to vehicle-injected and trained littermates (Figure 1C; Table 1C).

A functional significance of the compensatory increase of PKCι in LTP was tested. PKCι-null mice are embryonically lethal (*Seidl et al., 2013*), and therefore the effect of PKCι gene ablation on LTP has been studied by locally expressing Cre-recombinase in the hippocampus of PKCι$^{fl/fl}$ mice (*Sheng et al., 2017*). PKCι was ablated using Cre-expressing adeno-associated virus (AAV) in one hippocampus of PKCι-floxed/PKMζ-null mice (*Prkci$^{fl/fl}$-Prkcz$^{-/-}$*) (Figure 2). The contralateral hippocampus was injected with a control AAV-eGFP, and *ex vivo* slices were prepared for electrophysiology. PKCι decreased in the ipsilateral hippocampus to ~20% compared to the contralateral hippocampus. High-frequency stimulation (HFS) of the Schaffer collateral/commissural-CA1 synapses in the ipsilateral ι/ζ-dKO slices produced no change in the residual PKCι and a transient LTP lasting ~1-2 h (Figures 2 and 3A). In contrast, HFS of the contralateral slices persistently increased PKCι to ~200% and produced a late-LTP lasting 3 h (Figures 2 and 3A), similar to the compensated LTP previously observed in PKMζ-null mice (*Tsokas et al., 2016*). In addition, slices from hippocampus injected with AAV expressing Cre by the CaMKIIα-promoter to selectively delete PKCι in excitatory neurons resulted in a similar transient LTP (Figure 3A, inset; Figure 3 — figure supplement 1).

We tested if late-LTP could be induced in ι/ζ-dKO hippocampus by increasing HFS from 2 trains, 10 sec apart, which is optimized to produce an early onset of late-LTP (*Tsokas et al., 2007*), to 4 trains, spaced 5 min apart, which is optimized to produce maximal late-LTP (*Scharf et al., 2002; Serrano et al., 2005*) (Figure 3B). Stronger stimulation induces LTP in ι/ζ-dKO slices that lasts ~1-2 h. This LTP was no longer expressed by 3 h post-stimulation at which time field excitatory postsynaptic potentials (fEPSPs) did not significantly differ from fEPSPs before HFS.

LTP production in ι/ζ-dKO slices allowed testing of the selectivity of the aPKC-inhibitor zeta-inhibitory peptide (ZIP) that mimics the autoinhibitory sequences of PKCζ and PKCι. ZIP blocks the potentiation of synaptic transmission by postsynaptically perfused PKMζ or PKCι, but not the potentiation caused by phorbol ester stimulation of the conventional/novel PKCs (*Tsokas et al., 2016*). ZIP disrupts LTP in slices from both WT and PKMζ-null mice (*Volk et al., 2013; Tsokas et al., 2016*). ZIP might also disrupt LTP by off-target effects independent of aPKC inhibition (*Sadeh et al., 2015; LeBlancq et al., 2016; Stokes et al., 2025*). If ZIP disrupts LTP by off-target effects, ZIP should inhibit LTP regardless of aPKC expression. ZIP application had no effect on ι/ζ-dKO-LTP, suggesting that ZIP disrupts LTP by targeting PKMζ or PKCι (Figure 3B).

We then used active place avoidance to examine spatial memory in mice with bilateral hippocampal ι/ζ-dKO. ι/ζ-dKO mutant mice received three daily 30-min training trials and long-

term memory retention was tested without shock 1 day later (Figure 4A). Compared to control *PKCι*$^{fl/fl}$-*PKMζ*$^{-/-}$ littermates bilaterally injected in hippocampus with AAV-eGFP, mice with ι/ζ-dKO hippocampus show intact learning and short-term active place avoidance memory (Figure 4B), but defective long-term memory retention in which avoidance was not significantly different from pretraining (Figure 4C).

Discussion

The main finding of this study is the critical requirement of PKCι for compensatory restoration of hippocampal LTP and spatial memory in PKMζ-KO mice. In PKMζ-null mice, LTP is eliminated in medial prefrontal cortex (*Kniffin et al., 2025*) yet present in hippocampus (*Volk et al., 2013; Tsokas et al., 2016*). Nonetheless, in WT animals hippocampal LTP is disrupted by shRNA knockdown of PKMζ mRNA (*Wang et al., 2016*), PKMζ-antisense oligodeoxynucleotides (*Tsokas et al., 2016*), PKMζ-selective inhibitors (*Tsokas et al., 2024*), and dominant-negative PKMζ (*Ling et al., 2002*). A hallmark of genetic compensation is the effectiveness of knockdowns to yield a phenotype while knockouts do not (*El-Brolosy and Stainier, 2017*). Genetic compensation occurs when a paralog can functionally replace the mutated gene (*Gu et al., 2003; Conant and Wagner, 2004; White et al., 2013; El-Brolosy and Stainier, 2017*). Here we provide evidence that PKMζ's paralog, PKCι, is necessary to compensate for the hippocampal loss of PKMζ. PKMζ compensates for the loss of PKCι after hippocampal ablation (*Sheng et al., 2017*), suggesting that the aPKCs mutually compensate to sustain hippocampal LTP.

In contrast to KOs of the individual aPKCs, dKO of both PKMζ and PKCι disrupts hippocampal late-LTP (Figure 3). Induction of hippocampal LTP by either massed or spaced HFS produces early-LTP lasting 1-2 h, but late-LTP lasting >3 h is eliminated or severely diminished. In WT mice, PKCι is a key mediator of early-LTP (*Ren et al., 2013; Wang et al., 2016*); therefore, residual ZIP-insensitive early hippocampal LTP in dKO mice suggests that additional compensatory mechanisms can help mediate early-LTP independent of aPKCs. PKCβI levels increase in both constitutive and conditional PKMζ-KO mice (*Tsokas et al., 2016*) (Figure 1B, Table 1B); this increased expression of PKCβI may contribute to the aPKC-independent early-LTP. These findings highlight that LTP has distinct molecular mechanisms that underlie a multi-phase process for induction, early-, and late-phases occurring within seconds, 30 minutes, and 3 hours, respectively. LTP induction does not require aPKCs, instead CaMKIIα is crucial (*Tullis et al., 2023; Bayer and Giese, 2024*). Early-LTP does not require new protein synthesis but requires PKCι (*Ren et al., 2013; Wang et al., 2016*). In contrast, late-LTP requires *de novo* protein synthesis of PKMζ (*Osten et al., 1996; Tsokas et al., 2016*). Synthesis of other proteins are likely required as well.

ZIP, which inhibits synaptic potentiation by PKMζ and PKCι, but not by the phorbol ester activation of conventional/novel PKCs (*Tsokas et al., 2016*), does not affect ι/ζ-dKO-LTP (Figure 3B). This indicates that aPKCs are essential for the ability of ZIP to disrupt LTP in WT and PKMζ-KO mice. These pharmacogenetic experiments argue against potential off-target effects of ZIP for LTP inhibition (*Sadeh et al., 2015; LeBlancq et al., 2016; Stokes et al., 2025*). The possibility of non-specific effects of ZIP were investigated in the context of endogenous ζ and ι expression. Our experiments suggest that some so-called off-target effects likely result

from aPKC inhibition, particularly if the experimental outcome depends upon the postsynaptic AMPAR-trafficking pathway by which PKMζ potentiates synaptic transmission (*Yao et al., 2008; Stokes et al., 2025*).

Bilateral hippocampal ι/ζ-dKO eliminates long-term spatial memory without affecting learning, short-term memory, or expression of the place avoidance behavior (Figure 4). Memory deficits were also observed after conditional KO of both aPKCs selectively in neurons (*Scott et al., 2019*). Deletion of both aPKCs produce memory deficits for simple behaviors (Figure 4) (*Scott et al., 2019*); this contrasts with the specific deficits for cognitively demanding tasks when PKMζ or PKCι genes are individually deleted (*Tsokas et al., 2016; Sheng et al., 2017*). The results with PKMζ-null mice suggest that hippocampal LTP, which can be maintained by either aPKC, could subserve simple tasks, whereas complex tasks require neocortical LTP that can only be mediated by PKMζ (*Tsokas et al., 2016; Kniffin et al., 2025*).

How does PKCι compensate for PKMζ to maintain hippocampal LTP and long-term memory? Compensation can be induced after gene knockout by the fragments of mRNA that are produced by transcription upstream of the site of Cre-recombination (*El-Brolosy and Stainier, 2017; El-Brolosy et al., 2019; Ma et al., 2019*). This could explain why PKMζ-KOs produce compensation, but alterations that retain full-length PKMζ mRNA expression do not. In medial prefrontal cortex of PKMζ-KO mice, both early- and late-LTP are absent (*Kniffin et al., 2025*). This suggests that the PKCι activation that mediates early-LTP in the WT hippocampus may not be present to compensate for loss of PKMζ in ζ-KO medial prefrontal cortex.

Compensation for PKMζ function by PKCι in hippocampus likely requires additional posttranslational mechanisms to persistently activate and localize PKCι at active synapses (Figure 1C). PKMζ is persistently active because it possesses an independent catalytic domain that lacks the PKCζ autoinhibitory regulatory domain (*Sacktor et al., 1993*). PKCι has a regulatory domain that inhibits its catalytic domain, but the kinase can be activated by displacement of its regulatory domain by binding to postsynaptic proteins such as p62 (*Jiang et al., 2009; Ren et al., 2013*). This activation of PKCι by protein-protein interaction may sustain kinase activity longer than the transient second-messenger activation of the conventional/novel PKCs, and allow PKCι to replace PKMζ for maintenance. In addition to PKMζ's autonomous activity, the maintenance of persistent PKMζ action in LTP and memory requires binding to the postsynaptic scaffolding protein KIBRA/WWC1 (kidney and brain protein/WW and C2 domain protein 1) (*Tsokas et al., 2024; Shouval et al., 2025*). This interaction localizes PKMζ to active synapses in a process called persistent synaptic tagging. KIBRA also binds PKCι, albeit more weakly than PKMζ (*Tsokas et al., 2024*). In WT mice, the stronger binding of PKMζ to KIBRA might allow it to replace PKCι at active synapses in the transition from early- to late-LTP. In PKMζ-KO mice, PKCι at active synapses would not be replaced. PKMζ and PKCι also compete for binding to PAR3 (partitioning defective protein 3), another postsynaptic protein that localizes aPKCs within neurons (*Parker et al., 2013; Zhang and Wei, 2022*). Thus, when PKMζ is genetically deleted, persistent synaptic tags such as KIBRA and PAR3 become available for PKCι to maintain hippocampus-dependent LTP and long-term memory.

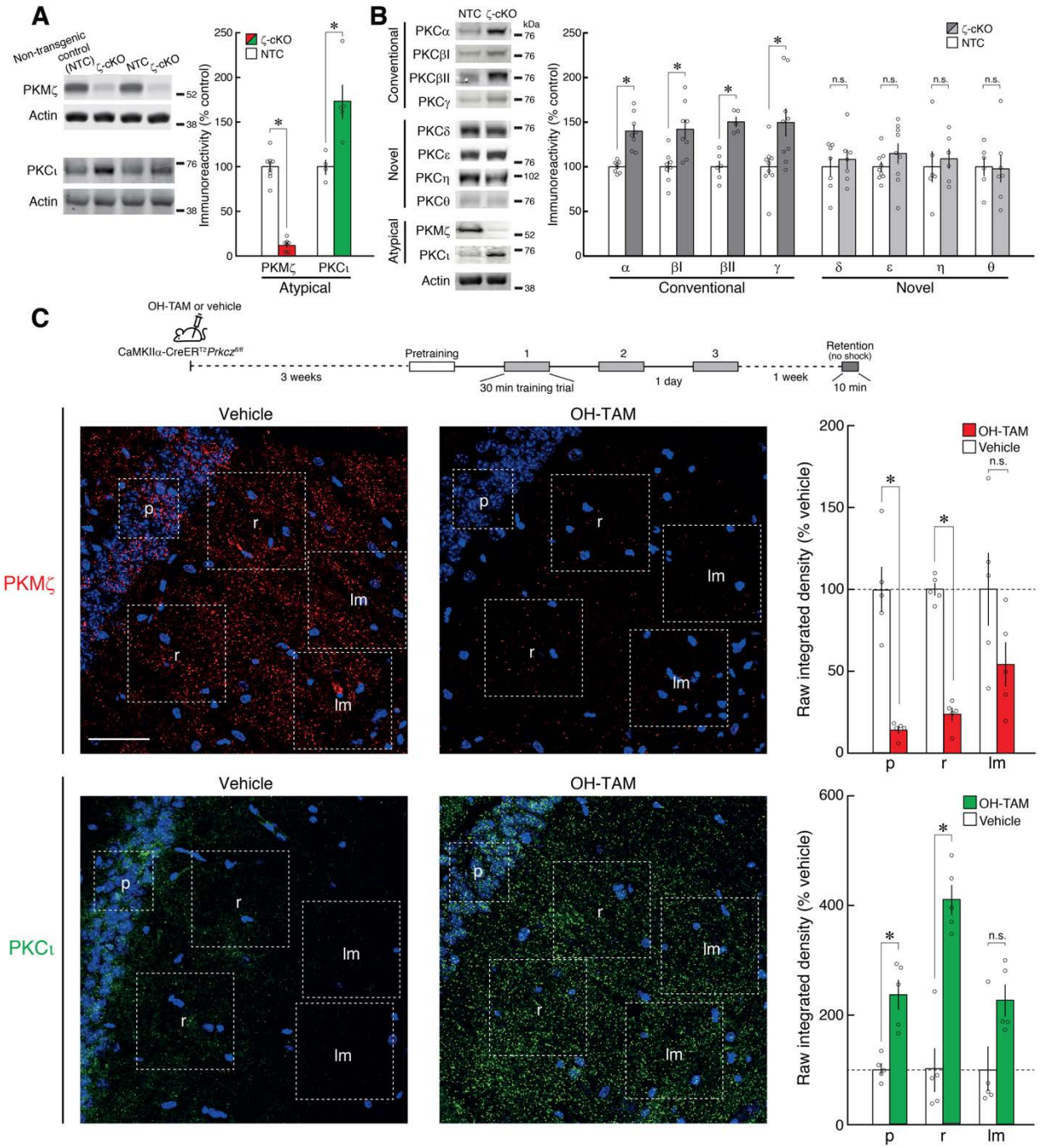

Figure 1. Compensatory increases of PKCι and conventional PKCs in the hippocampus of conditional PKMζ-KO mice. (**A, B**) Immunoblots of hippocampus from *Camk2a-CreER^{T2}Prkcz$^{fl/fl}$* mice receiving tamoxifen (2 mg/200 µl i.p., 5 daily doses) to activate Cre recombinase selectively in excitatory neurons. Mice are sacrificed 7 days after the last dose. Tamoxifen is a partial PKC antagonist and may still be present after a week (*O'Brian et al., 1985*). Therefore, WT mice that also received tamoxifen are non-transgenic controls (NTC). (**A**) PKMζ decreases and PKCι increases in PKMζ-cKO mice. Left, representative immunoblots with Mr markers. Right, mean ± SEM. Significance by two sample Student *t* tests with

Bonferroni correction denoted by *; not significant, n.s.; statistics in Table 1A. (**B**) Conventional PKCs increase and novel PKCs do not change; statistics in Table 1B. For clarity, the actin shown is for PKCs α, ι, and PKMζ immunoblots. (**C**) Immunohistochemistry shows PKMζ ablation reduces PKMζ and increases PKCι in CA1 *st. pyramidale* (p) and *radiatum* (r), but not *lacunosum-moleculare* (lm). Inset above, schematic of experimental protocol. PKMζ is deleted using *Camk2a-CreER*^{T2}*Prkcz*$^{fl/fl}$ mice. Cre is activated using 4-OH tamoxifen (OH-TAM, 2 mg/200 µl in sunflower seed oil i.p., 3 doses every other day). Control mice receive vehicle injections. Active place avoidance training begins 3 weeks later, and 1 week after training memory retention is tested in the absence of shock. Left above, immunohistochemistry reveals reduced PKMζ expression in cell bodies and dendritic compartments in PKMζ-cKO. Left below, PKCι increases in cell bodies and in dendritic compartments where it is ordinarily expressed at low levels. Right, mean ± SEM. Student t tests with Bonferroni corrections compared differences in PKMζ and PKCι expression separately in *strata* of CA1 (Table 1C). DAPI staining of nuclei shown in blue. Bar = 50 µm.

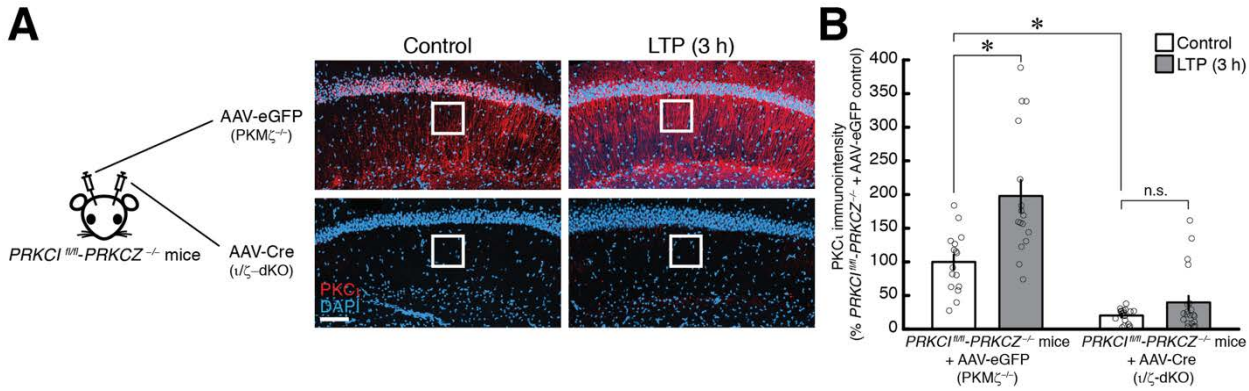

Figure 2. Compensatory increases of PKCι during hippocampal late-LTP maintenance in *Prkci*$^{fl/fl}$-*Prkcz*$^{-/-}$ mice. (A) Left, schematic of experimental protocol shows AAV expressing Cre by CMV promoter injected into ipsilateral hippocampus of a *Prkci*$^{fl/fl}$-*Prkcz*$^{-/-}$ mouse, and control AAV expressing eGFP into contralateral hippocampus. Hippocampal slices are prepared 3 weeks later. Right, representative images of PKCι-immunohistochemistry from adjacent slices in AAV-eGFP-injected, ζ-KO hippocampus show PKCι persistently increases 3 h post-tetanization (top row), and low and unchanging levels of PKCι in the ι/ζ-dKO hippocampus (bottom row). White boxes show *st. radiatum* regions of interest. **(B)** Mean ± SEM. The two-way ANOVA reveals the main effects of treatment (AAV-Cre [ι/ζ-dKO] vs. AAV-eGFP [ζ-KO], $F_{1,68} = 83.58$, $P < 0.00001$, $\eta^2_p = 0.55$), and stimulation (HFS vs. test, $F_{1,68} = 20.47$, $P = 0.00003$, $\eta^2_p = 0.23$), and an interaction of treatment x stimulation ($F_{1,68} = 9.09$, $P = 0.004$, $\eta^2_p = 0.12$). *Post-hoc* analysis confirms that, compared to the control group of ζ-KO, the intensity of PKCι immunoreactivity was significantly decreased in ι/ζ-dKO (*P*'s < 0.002 for both control and LTP in ι/ζ-dKO), yet increased in ζ-KO after LTP (P = 0.00011, ζ-KO, n's = 16, ι/ζ-dKO, n's = 20). Intensity of PKCι immunoreactivity did not change in the ι/ζ-dKO between the control and LTP groups (*P* = 0.3). Bar = 100 µm.

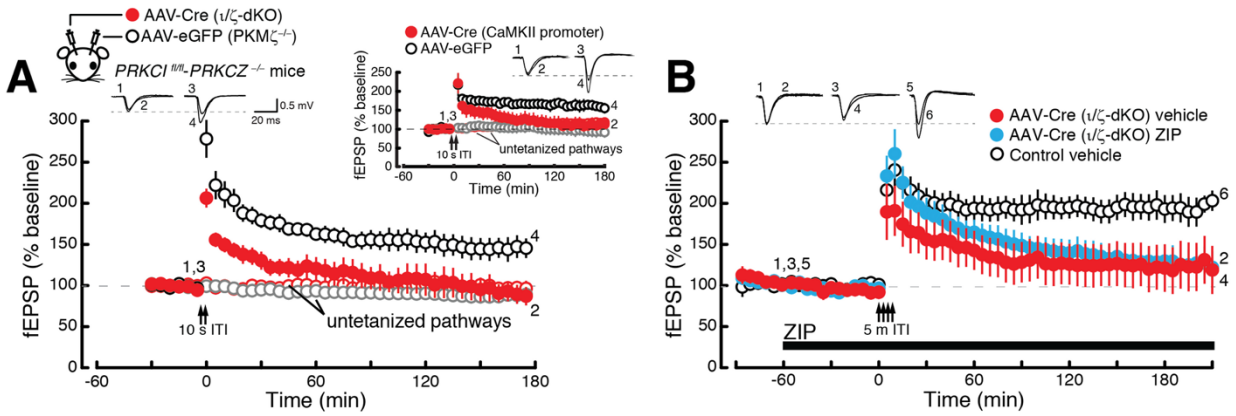

Figure 3. ι/ζ-dKO hippocampus shows transient, ZIP-insensitive LTP and no late-LTP. (**A**) Late-LTP is absent in ι/ζ-dKO hippocampus. Above left inset, schematic of intrahippocampal injections of AAC-Cre and AAV-eGFP. Middle inset, representative fEPSPs correspond to numbered times in the time-course below. Below, filled red circles, AAC-Cre expressed by CMV promoter and HFS; open red circles, test stimulation within the hippocampal slice. HFS tetani shown at arrows. Open black circles, AAV expressing eGFP with HFS; open grey circles, with test stimulation. Three-way mixed-design ANOVA reveals main effects of treatment (hippocampal injections of AAV-Cre [ι/ζ-dKO] vs. AAV-eGFP [ζ-KO], $F_{1,20} = 8.45$, $P = 0.0009$, $\eta^2_p = 0.30$), and stimulation (HFS vs. test stimulation, $F_{1,20} = 5.90$, $P = 0.025$, $\eta^2_p = 0.23$), as well as a 3-way interaction among treatment x stimulation x time (5-min average of pre-HFS and 3-h post-HFS, $F_{1,20} = 12.68$, $P = 0.002$, $\eta^2_p = 0.39$). *Post-hoc* analysis confirms established LTP is not maintained in ι/ζ-dKO 3 h after HFS when compared to pre-HFS basal responses ($P = 0.7$). *Post-hoc* analysis also confirms the control hippocampus maintains established LTP ($P = 0.0002$). Test stimulation was unaffected by Cre and eGFP AAV injections ($P = 0.9$ and $P = 0.7$, respectively, n's = 6). Right inset, ι/ζ-dKO by CaMKIIα promoter expression of Cre recombinase eliminates late-LTP. Three-way mixed-designed ANOVA reveals interaction between treatment (ζ-KO vs. ι/ζ-dKO) and stimulation (HFS vs. test stimulation, $F_{1,14} = 6.62$, $P = 0.02$, $\eta^2_p = 0.32$), and a 3-way interaction among treatment, stimulation, and time (5 min pre-HFS and 3 h post-HFS, $F_{1,14} = 8.56$, $P = 0.01$, $\eta^2_p = 0.38$). *Post-hoc* analysis confirms that compared to pre-HFS basal responses, LTP is not maintained in ι/ζ-dKO hippocampus 3 h post-HFS ($P = 0.8$) and is maintained in the control hippocampus ($P = 0.003$). AAV injections did not affect test stimulation ($P = 0.4$ and $P = 0.9$, respectively). ι/ζ-dKO HFS, n = 5; ι/ζ-dKO test, n = 4, ζ-KO HFS, n = 5, ζ-KO test, n = 4. (**B**) ZIP (5 μM in the bath) has no effect on ι/ζ-dKO-LTP induced by multiple spaced tetanic trains. ANOVA with repeated measurement reveals main effects of time (5 min pre-ZIP, 5 min pre-HFS, 20 min post-HFS, and 3 h post-HFS, $F_{3,24} = 26.91$, $P < 0.0001$, $\eta^2_p = 0.77$). Post-hoc analysis confirms ZIP did not alter the basal responses of ι/ζ-dKO (5 min pre-ZIP vs. 5 min pre-HFS, $P = 0.9$). Early-LTP is established in all 3 groups (5 min pre-HFS vs. 20 min post-HFS, in ι/ζ-dKO with vehicle, $P = 0.005$, ι/ζ-dKO with ZIP, $P = 0.005$, control, $P = 0.001$), and there is no difference between ι/ζ-dKO with and without ZIP at 20 min post-HFS ($P = 0.94$). However, LTP in ι/ζ-dKO did not persist 3 h with or without ZIP (5 min pre-HFS vs. 3 h post-HFS, $P = 0.8$ and 0.6, respectively), whereas LTP persists for 3 h in control with vehicle ($P = 0.008$). The ι/ζ-dKO with vehicle, n = 5; ι/ζ-dKO with ZIP, n = 3; control, n = 4.

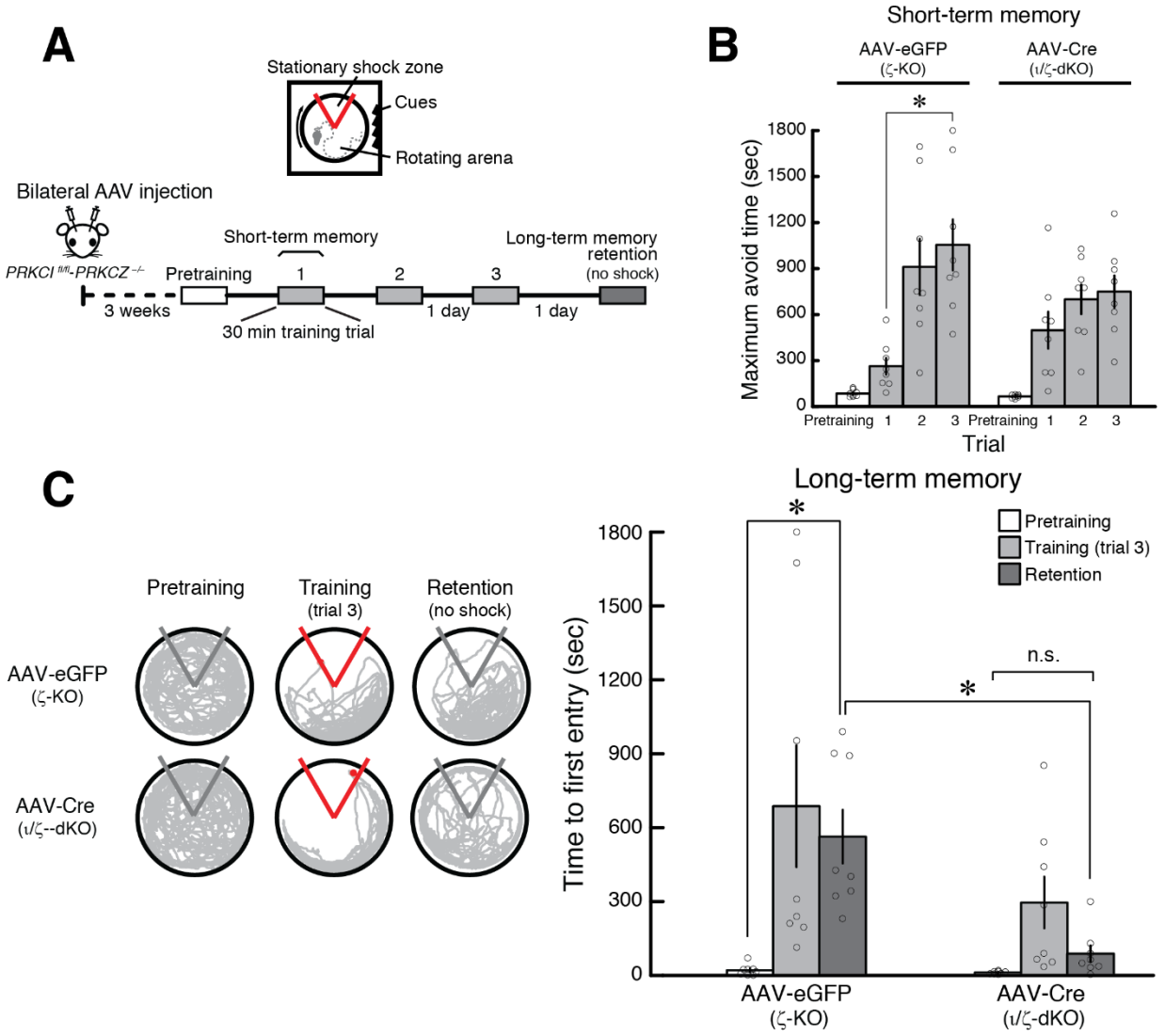

Figure 4. Intact spatial short-term memory and impaired long-term memory in mice with bilateral hippocampal ι/ζ-dKO. (**A**) Above, schematic of active place avoidance training apparatus shows a slowly rotating arena containing a nonrotating shock zone sector (delineated in red). Visual cues located on the walls of the room are needed to avoid the shock zone. Below, protocol for active place avoidance. *Prkci*$^{fl/fl}$-*Prkcz*$^{-/-}$ mice are injected bilaterally in hippocampus with AAV-Cre or AAV-eGFP (control). (**B**) ι/ζ-dKO does not affect short-term memory as assessed by maximum avoidance time during the first training trial. The contrast analysis reveals that the increases of maximum avoidance time from pretraining to trial 1 are not different between AAV-eGFP-injected and AAV-Cre-injected groups (t_{14} = 1.91, P = 0.08, d = 1.91), indicating both groups of mice successfully established short-term memory. However, the improvement of maximum avoidance time from trial 1 to trial 3 are different between the groups (t_{14} = 2.93, P = 0.01, d = 2.88), suggesting the two groups performed differently between daily training sessions. In addition, the ANOVA with repeated measurement discovers no group effect (AAV-eGFP-injected vs. AAV-Cre-injected, $F_{1,14}$ = 0.56, P = 0.47, η^2_p = 0.04), but significant

effects of trial ($F_{3,42}$ = 30.37, P < 0.0001, η^2_p = 0.68), and interaction ($F_{3,42}$ = 2.93, P = 0.04, η^2_p = 0.17). *Post-hoc* tests confirm that the maximum avoidance time in trial 1 is not different between the two groups (P = 0.14). In addition, the AAV-eGFP-injected group improved their performance between trial 1 and trial 3 (P = 0.0002), whereas the AAV-Cre showed no improvement (P = 0.2; n's = 8). These data indicate no differences in short-term memory between AAV-eGFP- and AAV-Cre-injected groups, but only the AAV-Cre-injected group failed to improve between daily trials, suggesting inability to retain avoidance memory across days. (**C**) PKCι ablation impairs long-term memory in *Prkci*$^{fl/fl}$-*Prkcz*$^{-/-}$ mice. Left, representative paths during 10-min of pretraining, at end of training trial 3, and 1-day memory retention. Right, mean ± SEM. The ANOVA with repeated measurement finds main effects of group (AAV-eGFP vs. AAV-Cre, $F_{1,14}$ = 10.53, P = 0.006, η^2_p = 0.43) and training phase (pretraining, trial 3 of training, retention, $F_{2,28}$ = 7.65, P = 0.002, η^2_p = 0.35). *Post-hoc* analysis reveals that the mice with AAV-Cre-injected ι/ζ-dKO hippocampus perform poorer during the memory retention test, compared to AAV-eGFP-injected (P = 0.02). In addition, the mice with ι/ζ-dKO hippocampus show no difference between the memory retention test and pretraining trial (P = 0.9), whereas the AAV-eGFP-injected mice show long-term memory is maintained (P = 0.02; n's = 8).

Isozyme / region	Non-transgenic control (NTC)		ζ-cKO		Degree of freedom	*t*	*P*	Cohen's *d*
	Mean ± SEM	n	Mean ± SEM	n				
A.								
aPKC								
PKMζ	100 ± 4.9	9	11.8 ± 2.4	8	15	15.4	< 0.0001	7.5
PKCι	100 ± 7.3	4	172.4 ± 18.4	5	7	3.9	0.01	2.2
B.								
cPKC								
α	100 ± 2.3	8	140.0 ± 7.0	8	14	5.4	< 0.0001	2.7
βI	100 ± 6.2	8	142.1 ± 10.6	8	14	3.4	0.004	1.7
βII	100 ± 6.3	6	150.2 ± 5.5	5	9	5.9	0.0002	3.6
γ	100 ± 8.6	9	149.6 ± 15.4	9	16	2.8	0.01	1.3
nPKC								
δ	100 ± 10.4	7	108.2 ± 10.3	7	12	0.6	0.6	0.3
ε	100 ± 5.3	9	114.8 ± 11.0	9	16	1.2	0.2	0.6
η	100 ±17.1	6	109.0 ± 10.9	6	10	0.4	0.7	0.3
θ	100 ± 10.1	6	97.7 ± 14.9	7	11	0.1	0.9	0.1
C.								
PKMζ								
pyramidale	100 ± 13.6	5	14.1 ± 2.1	5	8	6.2	0.0003	3.9
radiatum	100 ± 3.6	5	23.6 ± 3.9	5	8	14.4	<0.0001	9.1
lac-mol	100 ± 22.0	5	54.2 ± 13.2	5	8	1.8	0.1	1.1
PKCι								
pyramidale	100 ± 9.8	5	235.3 ± 26.4	5	8	4.8	0.001	3.0
radiatum	100 ± 36.9	5	411.2 ± 26.2	5	8	6.9	0.0001	4.3
lac-mol	100 ± 40.1	5	226.7 ± 26.7	5	8	2.6	0.03	1.7

Table 1. Statistics of data presented in Figure 1. Significant differences with Bonferroni correction are highlighted in yellow.

Kinase	Mean ± S.E.M.	n	Mean ± S.E.M.	n	Degree of freedom	*t*	*P*	Cohen's *d*
A								
p-PKC								
p-PKCι	100 ± 4.0	7	181.1 ± 19.8	7	12	4.0	0.002	2.1
p-cPKC	100 ± 7.1	11	151.4 ± 16.4	11	20	3.3	0.009	1.2
p-PKCε	100 ± 11.7	9	104.0 ± 14.2	8	15	0.2	0.8	0.1
B								
CaMKII	100 ± 7.63	7	96.33 ± 1.95	6	11	0.43	0.67	0.24
p-CaMKII	100 ± 9.65	7	114.94 ± 11.17	7	12	1.01	0.33	0.54

Table 2. Statistics of data presented in Figure 1 — figure supplement 1. Significant differences with Bonferroni correction are highlighted in yellow.

Antibodies for immunoblotting	Vendor and catalog number	Concentration
aPKC		
PKMζ (C2)	Sacktor Lab (*Hernandez et al., 2003*)	1:20,000
PKCι (E-7)	Santa Cruz sc-376344	1:200
p-PKC		
p-T410 PKCζ (H2)	Santa Cruz sc-271962	1:200
Phospho-PKC (pan) (ζ Thr410) (190D10)	Cell Signaling Technology #2060	1:100
cPKC		
α (H-7)	Santa Cruz sc-8393	1:200
βI (E-3)	Santa Cruz sc-8049	1:500
βII (F-7)	Santa Cruz sc-13149	1:100
γ (C-4)	Santa Cruz sc-166385	1:200
n-PKC		
δ [EPR17075]	Abcam ab182126	1:100
ε (E-5)	Santa Cruz sc-1681	1:1000
η [EPR18513]	Abcam ab179524	1:200
θ (E-7)	Santa Cruz sc-1680	1:100
Other		
CaMKIIα	ThermoFisher (Invitrogen) 137300	1:100
p-CaMKII [T286]	Cell Signaling Technology #3361	1:100
Actin	Sigma A4700	1:5000

Antibodies for immunohistochemistry		
PKCι (E-7)	Santa Cruz sc-376344	1:500
PKMζ (C2)	Sacktor Lab (*Hernandez et al., 2003*)	1:8000

Table 3. Antibodies used for immunoblotting and immunohistochemistry.

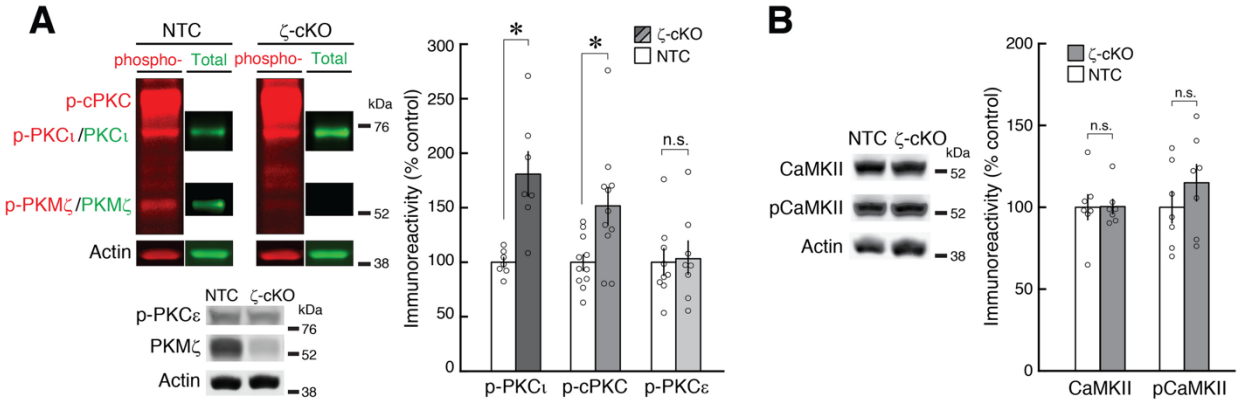

Figure 1 — figure supplement 1. ζ-cKO increases activation-loop-phosphorylation state of PKCι and cPKCs, but not novel PKCε or CaMKII autophosphorylation. (**A**) Left, above, representative immunoblots show increases in activation-loop phosphorylated PKCι (p-PKCι) and conventional-PKCs (p-cPKCs) in PKMζ-cKO mice. Red, phospho-PKCs; green, total PKCs from same samples. Below, p-PKCε, recognized by its higher Mr, does not change. Right, mean ± SEM (**B**) Levels of total CaMKIIα and T286-autophosphorylated CaMKIIα do not change in ζ-cKO hippocampus. Statistics in Table 2B.

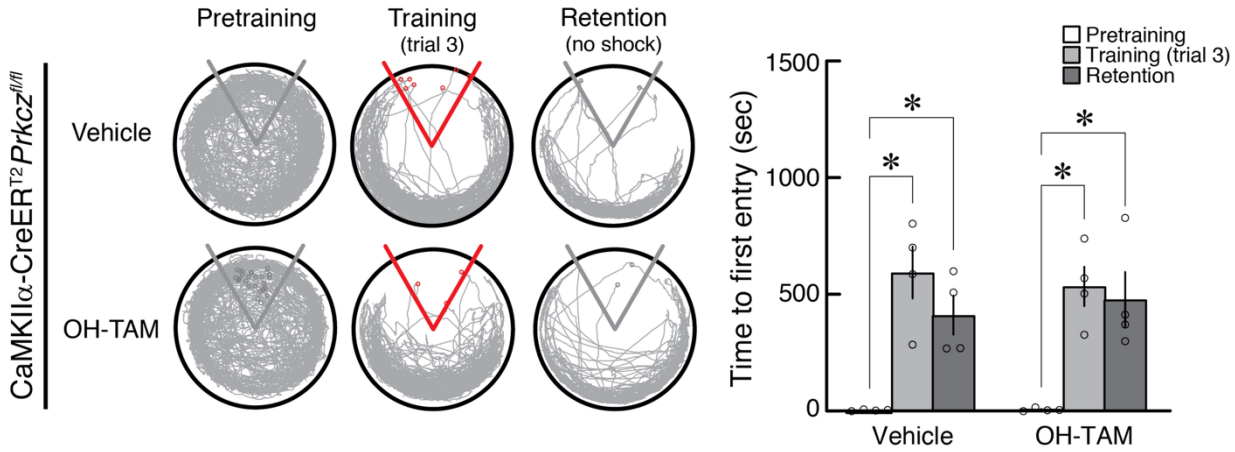

Figure 1 — figure supplement 2. Compensation for spatial memory in ζ-cKO mice. Two-way ANOVA (treatment X training) revealed a significant effect of training ($F_{1.662,\ 9.972} = 41.93$, $P < 0.0001$) but not an effect of treatment ($F_{1,\ 6} < 0.001$, $P = 0.97$) or their interaction ($F_{2,\ 12} = 0.48$, $P = 0.62$). Further comparisons using Bonferroni-corrected tests revealed significant differences between pretraining and training (Trial 3) ($P = 0.002$) and pretraining and retention ($P = 0.001$), but no differences between training (Trial 3) and retention ($P = 0.1$). Moreover, comparison also revealed significant differences between pretraining and training (Trial 3) in both vehicle and 4-OH tamoxifen groups ($P = 0.02$ and $P = 0.01$, respectively) and between pretraining and retention in both vehicle and 4-OH tamoxifen groups ($P = 0.03$ and $P = 0.05$, respectively), confirming the treatment groups did not behave differently in the training.

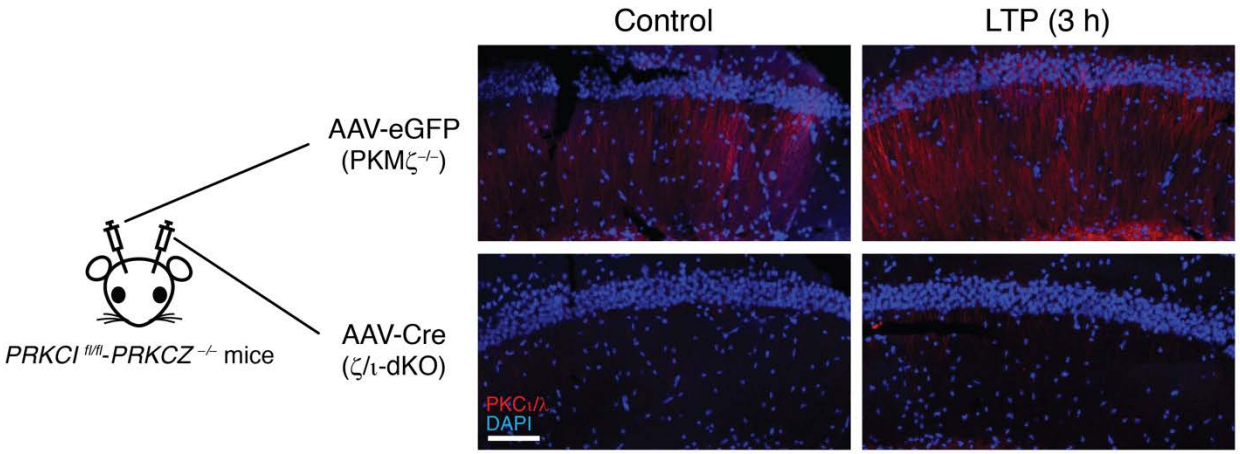

Figure 3 — figure supplement 1. Representative immunohistochemistry of AAV expressing Cre-recombinase by CaMKIIα promotor shows loss of PKCι in ι/ζ-cKO hippocampus and compensatory increase in PKCι during LTP maintenance of control eGFP-injected hippocampus. Left, schematic of sites of injection; right, PKCι immunohistochemistry. DAPI stains nuclei in CA1. Bar = 100 µm.

Methods:

Reagents, immunoblots (using antibodies described in Table 3), hippocampal slice preparation and recording, and active place avoidance behavior were as previously described (*Tsokas et al., 2016*). Male mice on C57BL/6 background at least 4 months old were used. The PKMζ-null mouse line was previously described (*Lee et al., 2013*) and provided by Robert O. Messing (Univ Texas at Austin, TX). Conditional PKMζ and PKCι mice were generated by Sourav Ghosh as previously described (*Scott et al., 2019; El Allam et al., 2024; Mercau et al., 2024*). *Camk2a-CreER*^{T2} mice were from Jackson Labs. AAVs expressing Cre-recombinase and eGFP were from Addgene. Immunohistochemistry for Figure 2 and Figure 3 — figure supplement 1 was as described (*Hsieh et al., 2021; Tsokas et al., 2024*), using primary antibody rabbit anti-PKCι (1:1,000, Cell Signaling #2998S) and in Table 3.

Immunohistochemistry for Figure 1C was performed as follows. Free-floating sections were permeabilized with phosphate-buffered saline (PBS) containing 0.1% Tween20 (PBS-T) for 1 h at room temperature and blocked with 10% normal goat serum in PBS-T (blocking buffer) for 2.5 h at room temperature. One batch of sections was then incubated overnight at 4 °C with primary antibody rabbit anti-PKMζ C-2 antisera (1:1,000) (*Hernandez et al., 2003*) and another batch of sections with rabbit anti-PKCι (1:1,000, Cell Signaling #2998S) in blocking buffer. After washing 3 times for 10 min each in PBS-T, both batches of sections were incubated with the secondary antibody goat anti-rabbit-Alexa 647 (1:500 in blocking buffer; Jackson ImmunoResearch) for 2 h at room temperature. After washing 3 times for 10 min each in PBS-T and extensive washing with PBS, the sections were mounted with Vectashield with 4′,6-diamidino-2- phenylindole (DAPI, Vector Laboratories). Three sections from each mouse were examined using an upright Leica SP8 confocal microscope and analyzed using ImageJ (version 1.53a). For each section, 8.5 µm-thick Z-stacks of the dorsal CA1 were created using the maximum intensity projection function in ImageJ. For each *st. pyramidale*, *radiatum*, and *lacunosum-moleculare*, two square regions of interest were centered in each stack. Measurements were made from each mouse in each region of interest. The raw integrated density (defined as the sum of the values for all pixels) of the Z-stack region of interest expressing the fluorescent label was measured for the volume of target pixels, and the average of each measurement was taken as representative for the region and each mouse. These measures account for the locations of the signal as well as their intensity.

AAV injections: Mice were anesthetized in a closed chamber filled with the inhalation anesthetic isoflurane (RWD Life Science, R510-22-10), and then fixed in a stereotaxic apparatus (Stoelting Co.). Anesthesia was maintained with isoflurane inhalation (1%−2.5% via trachea). The eyes of the mice were safeguarded using erythromycin ophthalmic ointment (0.5%). The skull was exposed and cleaned using 3% hydrogen peroxide. Small holes in the skull were then drilled with the following stereotaxic coordinates for each target site: left CA1 (triple injection: AP: -1/ ML: -0.7/ DV: -1.65; AP: -1.8/ ML: -1.5/ DV: -2; AP: -2.7/ ML: -2/ DV: -2, below the skull surface) and right CA1 (triple injection: AP: -1/ ML: +0.7/ DV: -1.65; AP: -1.8/ ML: +1.5/ DV -2; AP: -2.7/ ML: +2/ DV: -2, below the skull surface). The virus was injected using a 34-gauge needle with a Hamilton syringe at 0.1 µl/min rate into target regions. At all injected points, the tip of the needle was positioned 0.05 mm below the target coordinate and returned to the target site after 2 min. After injection, the needle stayed in place for an additional 7 min and was slowly withdrawn. For physiology, 0.5 ul of virus pENN.AAV.CMVs.PI.Cre.rBG (AAV2.9) (1 x 10^{13}

viral genomes [vg]/ml) was injected into one CA1, and 0.5 ul of virus pAAV.CMV.PI.EGFP.WPRE.bGH (AAV2.9) (1 x 10^{13} vg/ml) was injected in the contralateral side. Virus pENN.AAV.CamKIIa 0.4 Cre SV40 (AAV2.9) (1 x 10^{13} vg/ml) was used to express Cre-recombinase by the CaMKIIα promoter.

Statistics: Sample sizes vary for the different experimental approaches (biochemistry, extracellular field potential physiology, and behavior). The PKMζ is compensated hypothesis predicts all-or-none effects in the experiments, and this provided a basis for sample size estimates. Power analyses were performed using G*Power Version 3.1.9.7 with $\alpha = 0.05$ and $\beta = 0.8$ and large effect sizes of 1.5–2.0. The effect size estimates were based on prior studies that demonstrated essentially all-or-none effects of PKMζ inhibition on the immunoblot, immunohistochemical, physiological, and behavioral assays used here (*Tsokas et al., 2016; Hsieh et al., 2021; Tsokas et al., 2024*). Two-population Student's *t* tests with Bonferroni corrections were performed to compare protein levels by immunoblot and immunocytochemistry in the PKMζ-cKO and its appropriate control. For LTP experiments the responses to test stimuli were averaged across 5 min for statistical comparisons. Repeated measures ANOVA was used to compare the change in the potentiated response at the time points described. Multi-factor comparisons were performed using mixed-design ANOVA with repeated measures, as appropriate. The degrees of freedom for the critical *t* values of the *t* tests and the *F* values of the ANOVAs are reported as subscripts. *Post-hoc* multiple comparisons were performed by Newman-Keuls tests as appropriate. Statistical significance was accepted at $P < 0.05$. Effect sizes for binary comparisons and one-way ANOVAs are reported as Cohen's *d* and as η^2_p for multi-factor ANOVA effects.

References

Bayer KU, Giese KP. 2024. A revised view of the role of CaMKII in learning and memory. *Nat Neurosci*. 10.1038/s41593-024-01809-x.

Conant GC, Wagner A. 2004. Duplicate genes and robustness to transient gene knock-downs in Caenorhabditis elegans. *Proc Biol Sci* **271**: 89-96. 10.1098/rspb.2003.2560.

El Allam A, Alberto EJ, Mercau ME, Dimitrius T, Pramio DT, Krishna M, Bhat KM, Philbrick WM, Schechtman D, Rothlin CV et al. 2024. Functional roles of neural aPKCs in mouse brain development and survival. *bioRxiv:20240522595312*. doi: https://doi.org/10.1101/2024.05.22.595312.

El-Brolosy MA, Kontarakis Z, Rossi A, Kuenne C, Gunther S, Fukuda N, Kikhi K, Boezio GLM, Takacs CM, Lai SL et al. 2019. Genetic compensation triggered by mutant mRNA degradation. *Nature* **568**: 193-197. 10.1038/s41586-019-1064-z.

El-Brolosy MA, Stainier DYR. 2017. Genetic compensation: A phenomenon in search of mechanisms. *PLoS Genet* **13**: e1006780. 10.1371/journal.pgen.1006780.

Gu Z, Steinmetz LM, Gu X, Scharfe C, Davis RW, Li WH. 2003. Role of duplicate genes in genetic robustness against null mutations. *Nature* **421**: 63-66. 10.1038/nature01198.

Han J, Grau-Perales A, Harris RM, Kao H-Y, Pal A, Alarcon JM, Sacktor T, Martiniani S. ea. 2025. Persistently increased expression of PKMζ and unbiased gene expression profiles identify hippocampal molecular traces of a long-term active place avoidance memory and ‘shadow’ proteins. *bioRxiv* 2025.2004.2028.651143.

Hernandez AI, Blace N, Crary JF, Serrano PA, Leitges M, Libien JM, Weinstein G, Tcherapanov A, Sacktor TC. 2003. Protein kinase Mζ synthesis from a brain mRNA encoding an independent protein kinase Cζ catalytic domain. Implications for the molecular mechanism of memory. *J Biol Chem* **278**: 40305-40316. 10.1074/jbc.M307065200.

Hsieh C, Tsokas P, Grau-Perales A, Lesburgueres E, Bukai J, Khanna K, Chorny J, Chung A, Jou C, Burghardt NS et al. 2021. Persistent increases of PKMzeta in memory-activated neurons trace LTP maintenance during spatial long-term memory storage. *Eur J Neurosci* **54**: 6795-6814. 10.1111/ejn.15137.

Jiang J, Parameshwaran K, Seibenhener ML, Kang MG, Suppiramaniam V, Huganir RL, Diaz-Meco MT, Wooten MW. 2009. AMPA receptor trafficking and synaptic plasticity require SQSTM1/p62. *Hippocampus* **19**: 392-406. 10.1002/hipo.20528.

Kniffin A, English E, Briand L. 2025. PKMζ is necessary for LTD and LTP in the mPFC. *The Journal of physiology*. doi.org/10.1113/JP289373.

LeBlancq MJ, McKinney TL, Dickson CT. 2016. ZIP It: Neural Silencing Is an Additional Effect of the PKM-Zeta Inhibitor Zeta-Inhibitory Peptide. *J Neurosci* **36**: 6193-6198. 10.1523/JNEUROSCI.4563-14.2016.

Lee AM, Kanter BR, Wang D, Lim JP, Zou ME, Qiu C, McMahon T, Dadgar J, Fischbach-Weiss SC, Messing RO. 2013. Prkcz null mice show normal learning and memory. *Nature* **493**: 416-419. 10.1038/nature11803.

Ling DS, Benardo LS, Serrano PA, Blace N, Kelly MT, Crary JF, Sacktor TC. 2002. Protein kinase Mζ is necessary and sufficient for LTP maintenance. *Nat Neurosci* **5**: 295-296. 10.1038/nn829.

Ma Z, Zhu P, Shi H, Guo L, Zhang Q, Chen Y, Chen S, Zhang Z, Peng J, Chen J. 2019. PTC-bearing mRNA elicits a genetic compensation response via Upf3a and COMPASS components. *Nature* **568**: 259-263. 10.1038/s41586-019-1057-y.

Mercau ME, Hackbarth R-M, Liu X, Wang H, Zhang L, Basu MK, Rothlin CV, Ghosh S. 2024. Time-resolved function of cell polarity kinases PRKCZ and PRKCI in CNS myelination. *bioRxiv:20240422589759*.

Miller SG, Kennedy M. 1986. Regulation of brain type II Ca2+/calmodulin-dependent protein kinase by autophosphorylation: a Ca2+-triggered molecular switch. *Cell* **44**: 861-870.

O'Brian CA, Liskamp RM, Solomon DH, Weinstein IB. 1985. Inhibition of protein kinase C by tamoxifen. *Cancer research* **45**: 2462-2465.

Osten P, Valsamis L, Harris A, Sacktor TC. 1996. Protein synthesis-dependent formation of protein kinase Mζ in long-term potentiation. *J Neurosci* **16**: 2444-2451. not available.

Parker SS, Mandell EK, Hapak SM, Maskaykina IY, Kusne Y, Kim JY, Moy JK, St John PA, Wilson JM, Gothard KM et al. 2013. Competing molecular interactions of aPKC isoforms regulate neuronal polarity. *Proc Natl Acad Sci U S A* **110**: 14450-14455. 10.1073/pnas.1301588110.

Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC. 2006. Storage of spatial information by the maintenance mechanism of LTP. *Science* **313**: 1141-1144. 10.1126/science.1128657.

Ren SQ, Yan JZ, Zhang XY, Bu YF, Pan WW, Yao W, Tian T, Lu W. 2013. PKClambda is critical in AMPA receptor phosphorylation and synaptic incorporation during LTP. *The EMBO journal* **32**: 1365-1380. 10.1038/emboj.2013.60.

Sacktor TC, Osten P, Valsamis H, Jiang X, Naik MU, Sublette E. 1993. Persistent activation of the zeta isoform of protein kinase C in the maintenance of long-term potentiation. *ProcNatlAcadSciUSA* **90**: 8342-8346.

Sadeh N, Verbitsky S, Dudai Y, Segal M. 2015. Zeta Inhibitory Peptide, a Candidate Inhibitor of Protein Kinase Mzeta, Is Excitotoxic to Cultured Hippocampal Neurons. *J Neurosci* **35**: 12404-12411. 10.1523/JNEUROSCI.0976-15.2015.

Scharf MT, Woo NH, Lattal KM, Young JZ, Nguyen PV, Abel T. 2002. Protein synthesis is required for the enhancement of long-term potentiation and long-term memory by spaced training. *J Neurophysiol* **87**: 2770-2777.

Scott J, Thakar S, Mao Y, Qin H, Hejran H, Lee SY, Yu T, Klezovitch O, Cheng H, Mu Y et al. 2019. Apical-Basal Polarity Signaling Components, Lgl1 and aPKCs, Control Glutamatergic Synapse Number and Function. *iScience* **20**: 25-41. 10.1016/j.isci.2019.09.005.

Seidl S, Braun U, Roos N, Li S, Ludtke TH, Kispert A, Leitges M. 2013. Phenotypical analysis of atypical PKCs in vivo function display a compensatory system at mouse embryonic day 7.5. *PloS one* **8**: e62756. 10.1371/journal.pone.0062756.

Serrano P, Yao Y, Sacktor TC. 2005. Persistent phosphorylation by protein kinase Mζ maintains late-phase long-term potentiation. *J Neurosci* **25**: 1979-1984. 10.1523/JNEUROSCI.5132-04.2005.

Sheng T, Wang S, Qian D, Gao J, Ohno S, Lu W. 2017. Learning-Induced Suboptimal Compensation for PKCiota/lambda Function in Mutant Mice. *Cerebral cortex* **27**: 3284-3293. 10.1093/cercor/bhx077.

Shouval HZ, Hsieh C, Flores-Obando RE, Cano DA, Tracy TE, Sacktor TC. 2025. Maintenance of memory by negative feedback of synaptic protein elimination: modeling KIBRA-PKMzeta dynamics in LTP. *Learning & memory* **32**. 10.1101/lm.054077.124.

Stokes EG, Vasquez JJ, Azouz G, Nguyen M, Tierno A, Zhuang Y, Galinato VM, Hui M, Toledano M, Tyler I et al. 2025. Cationic peptides cause memory loss through endophilin-mediated endocytosis. *Nature*. 10.1038/s41586-024-08413-w.

Tsokas P, Hsieh C, Flores-Obando RE, Bernabo M, Tcherepanov A, Hernandez AI, Thomas C, Bergold PJ, Cottrell JE, Kremerskothen J et al. 2024. KIBRA anchoring the action of PKMzeta maintains the persistence of memory. *Sci Adv* **10**: eadl0030. 10.1126/sciadv.adl0030.

Tsokas P, Hsieh C, Yao Y, Lesburgueres E, Wallace EJ, Tcherepanov A, Jothianandan D, Hartley BR, Pan L, Rivard B et al. 2016. Compensation for PKMzeta in long-term potentiation and spatial long-term memory in mutant mice. *eLife* **5**: e14846. 10.7554/eLife.14846.

Tsokas P, Ma T, Iyengar R, Landau EM, Blitzer RD. 2007. Mitogen-activated protein kinase upregulates the dendritic translation machinery in long-term potentiation by controlling the mammalian target of rapamycin pathway. *J Neurosci* **27**: 5885-5894. 27/22/5885 [pii] 10.1523/JNEUROSCI.4548-06.2007.

Tullis JE, Larsen ME, Rumian NL, Freund RK, Boxer EE, Brown CN, Coultrap SJ, Schulman H, Aoto J, Dell'Acqua ML et al. 2023. LTP induction by structural rather than enzymatic functions of CaMKII. *Nature* **621**: 146-153. 10.1038/s41586-023-06465-y.

Volk LJ, Bachman JL, Johnson R, Yu Y, Huganir RL. 2013. PKM-zeta is not required for hippocampal synaptic plasticity, learning and memory. *Nature* **493**: 420-423. 10.1038/nature11802.

Wang S, Sheng T, Ren S, Tian T, Lu W. 2016. Distinct Roles of PKCiota/lambda and PKMzeta in the Initiation and Maintenance of Hippocampal Long-Term Potentiation and Memory. *Cell Rep* **16**: 1954-1961. 10.1016/j.celrep.2016.07.030.

White JK, Gerdin AK, Karp NA, Ryder E, Buljan M, Bussell JN, Salisbury J, Clare S, Ingham NJ, Podrini C et al. 2013. Genome-wide generation and systematic phenotyping of knockout mice reveals new roles for many genes. *Cell* **154**: 452-464. 10.1016/j.cell.2013.06.022.

Yao Y, Kelly MT, Sajikumar S, Serrano P, Tian D, Bergold PJ, Frey JU, Sacktor TC. 2008. PKMζ maintains late long-term potentiation by N-ethylmaleimide-sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors. *J Neurosci* **28**: 7820-7827. 28/31/7820 [pii] 10.1523/JNEUROSCI.0223-08.2008.

Zhang L, Wei X. 2022. The Roles of Par3, Par6, and aPKC Polarity Proteins in Normal Neurodevelopment and in Neurodegenerative and Neuropsychiatric Disorders. *J Neurosci* **42**: 4774-4793. 10.1523/JNEUROSCI.0059-22.2022.