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The CaMKII/GluN2B Protein Interaction Maintains Synaptic Strength[*]

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Learning, memory, and cognition are thought to require normal long-term potentiation (LTP) of synaptic strength, which in turn requires binding of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to the NMDA-type glutamate receptor (NMDAR) subunit GluN2B. For LTP induction, many additional required players are known. Here we tested the hypothesis that CaMKII/GluN2B binding also mediates the more elusive maintenance of synaptic strength. Intriguingly, the CaMKII inhibitor tatCN21 reduces synaptic strength only at high concentrations necessary for CaMKII/NMDAR disruption (20 μM) but not at lower concentrations sufficient for kinase inhibition (5 μM). However, increased concentration also causes unrelated effects. Thus, to distinguish between correlation and causality, we used a pharmacogenetic approach. In a mouse with a mutant NMDAR GluN2B subunit that is CaMKII binding-incompetent, any tatCN21 effects that are specific to the CaMKII/GluN2B interaction should be abolished, and any remaining tatCN21 effects have to be nonspecific (*i.e.* mediated by other targets). The results showed that the persistent reduction of synaptic strength by transient application of 20 μM tatCN21 had a nonspecific presynaptic component (on fiber volley amplitude) that was unrelated to the CaMKII/GluN2B interaction or CaMKII activity. However, the remaining component of the persistent tatCN21 effect was almost completely abolished in the GluN2B mutant mouse. These results highlight the requirement for stringent pharmacogenetic approaches to separate specific on-target effects from nonspecific off-target effects. Importantly, they also demonstrate that the CaMKII/GluN2B interaction is required not only for normal LTP induction but also for the maintenance of synaptic strength.

The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)[3] and the NMDA-type glutamate receptor (NMDAR) are central mediators of long-term potentiation (LTP) and depression (LTD), forms of synaptic plasticity thought to underlie learning, memory, and cognition (1; for a review, see Refs. 2–5). Many additional molecular players have been implicated in the induction of these changes of synaptic strength (4, 6, 7); however, the mechanisms responsible for then maintaining synaptic strength have been elusive. CaMKII has been hypothesized to mediate not only LTP induction but also the maintenance of synaptic strength (2–4), as Ca^{2+} influx through NMDARs triggers CaMKII autophosphorylation at Thr-286 to generate Ca^{2+}-independent "autonomous" kinase activity, a process that can provide "molecular memory" of the initial Ca^{2+} stimulus (8, 9). However, although CaMKII Thr-286 phosphorylation is indeed required for LTP (10), this requirement is for induction rather than maintenance. Thr-286 phosphorylation of synaptic CaMKII is reversed within 2 min of LTP induction (11), and CaMKII inhibition after LTP induction does not affect maintenance (9). Another kinase that has received prominent attention as a "memory kinase" is PKMζ, and its inhibitor myrZIP does indeed reverse LTP maintenance (12–14). However, PKC/PKMζ knockout mice have normal LTP, and, more importantly, this LTP is still completely reversed by myrZIP (15). Although normal LTP in knockout mice could be explained by compensatory effects, the pharmacogenetic combination showed that myrZIP reverses LTP by targets other than PKMζ. Thus, we decided to use a similar pharmacogenetic approach to test a remaining hypothesis for a mechanism that could maintain synaptic strength: the regulated binding of CaMKII to the NMDAR subunit GluN2B (for a review, see Refs. 2, 3).

CaMKII/GluN2B binding requires an initial Ca^{2+}/CaM stimulus but then persists even after dissociation of CaM from the complex. This also leaves CaMKII in a partially autonomous conformation and mediates the persistent CaMKII accumulation at synapses after LTP stimuli (16–21). Indeed, making GluN2B incompetent for CaMKII binding in GluN2BΔCaMKII knockin (KI) mice causes impaired LTP (20), although this does not distinguish between functions in LTP induction *versus* maintenance. However, transient treatment of hippocampal slices with high concentrations of the CaMKII inhibitor

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3 The abbreviations used are: CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; NMDAR, NMDA-type glutamate receptor; LTP, long-term potentiation; LTD, long-term depression; KI, knockin; ANOVA, analysis of variance; RMANOVA, repeated measures analysis of variance; FV, fiber volley; fEPSP, field excitatory postsynaptic potential; I/O, input/output.

tatCN21 (20 μM (22) persistently disrupts the CaMKII-NMDAR complex and persistently reduces synaptic strength both basally and after LTP (23). By contrast, lower concentrations of tatCN21 (5 μM) that do not disrupt the CaMKII-NMDAR complex (23) do not affect the maintenance of synaptic strength even though they are sufficient to completely block CaMKII activity and LTP induction (9). However, although these inhibitor findings provide an intriguing correlation, they do not show causation, as highlighted by the recent PKMζ/myrZIP example. Indeed, at 20 μM, but not at 5 μM, tatCN21 additionally promotes significant formation of unusual CaMKII/polyribosome aggregates (24), providing another mechanistic correlation. Additionally, although 5 μM tatCN21 (125$\times$ IC$_{50}$ for CaMKII (9)) affects the related CaMKIV only very mildly at best (22), a higher concentration would be expected to have a stronger effect. Furthermore, although the tatCN21 treatment that disrupts CaMKII/GluN2B binding affected both potentiated and basal transmission (23), GluN2BΔCaM KI mice showed normal basal transmission (20). However, the normal transmission in KI mice also enables a pharmacogenetic combination approach to distinguish between the two possible causes underlying the apparent discrepancy: the normal basal transmission in the KI mice may be caused by compensatory effects, or the persistent reduction in transmission by acute tatCN21 application may be caused by nonspecific effects unrelated to the CaMKII/GluN2B interaction.

Here we employed a pharmacogenetic approach that used tatCN21 in combination with GluN2BΔCaMKII mice. In these KI mice, any effects of tatCN21 that are mediated by CaMKII/GluN2B disruption should be eliminated. Any remaining tatCN21 effects could not be mediated by the CaMKII/GluN2B disruption (as there is no CaMKII/GluN2B interaction in these mice to begin with). The results showed that 20 μM tatCN21 has a strong persistent but nonspecific presynaptic effect (on fiber volley amplitude), further highlighting the requirement of pharmacogenetics for testing the specificity of drug treatments. However, the remaining persistent component of transient application of 20 μM tatCN21 was almost completely abolished in the GluN2BΔCaMKII mice. Thus, despite the nonspecific presynaptic effect of high tatCN21 concentrations detected here, the specificity of the remaining effect demonstrates that the CaMKII/GluN2B interaction indeed mediates maintenance of synaptic strength.

Results

Electrophysiological Effects of tatCN21 Mouse Hippocampal Slices—Application of 20 μM tatCN21 was observed previously to reduce synaptic strength with a partial recovery after washout in hippocampal slices of rats. To determine whether mice could similarly be utilized in such experiments, basal transmission was measured in the presence of tatCN21 in hippocampal slices from WT mice. As was observed previously in rats (23), 20 μM tatCN21 significantly reduced both basal and potentiated transmission in WT mice, which partially recovered after washout (Fig. 1). The 30-min treatment of 20 μM tatCN21 reduced basal transmission to ~10% baseline, with a recovery to ~30% after washout for 1 h. All changes in synaptic strength were

significant ($p < 0.001$, baseline *versus* drug and baseline *versus* washout; $p < 0.05$, drug *versus* washout; one-way RMANOVA; Fig. 1, *A* and *B*). These reductions followed the same pattern as seen previously in rats, although the effect sizes were more pronounced in mice. For potentiated slices, saturated LTP was reversed by 20 μM tatCN21, and this reversal persisted even after washout of the drug (Fig. 1*C*). After washout of tatCN21, LTP could be reinduced (Fig. 1*C*), indicating that the persistent reversal was not due to persistent effects on slice health and that the CaMKII activity required for LTP induction was no longer blocked after the washout. In contrast to 20 μM tatCN21, a treatment with 5 μM tatCN21 (which is sufficient to block CaMKII activity and LTP induction (9) did not cause any reduction in synaptic transmission (Fig. 1*D*), confirming that the reduction in maintenance of synaptic strength requires an effect of tatCN21 that is only seen at the higher concentration and is not dependent on blocking enzymatic activity of CaMKII.

20 μM tatCN21 Differentially Affects WT and GluN2BΔCaMKII Mice—To test the mechanistic hypothesis that the persistent reduction in synaptic strength by tatCN21 is a result of reversing CaMKII/GluN2B binding, tatCN21 treatment was also tested in slices from GluN2BΔCaMKII KI mice. In these KI mice, any tatCN21 effects that are specifically mediated by the disruption of the CaMKII/GluN2B interaction should be eliminated (Fig. 2*A*). Although these KI mice have reduced LTP, they display normal basal synaptic transmission (20). Thus, the effects of tatCN21 were tested only on basal synaptic strength. In these KI mice, 20 μM tatCN21 acutely induced an initial reduction that did not significantly recover (~45% baseline during treatment and ~55% baseline after 1 h of washout, Fig. 2*A*). Thus, in contrast to WT slices, the drug and washout phases did not significantly differ from each other in KI slices, although they were both still significantly reduced from baseline ($p < 0.001$, one-way RMANOVA, Fig. 2*C*). Compared with the WT slices, the reduction in the KI slices by 20 μM tatCN21 was significantly less during both drug treatment and washout ($p < 0.001$ and $p < 0.05$, respectively; two-way RMANOVA; Fig. 2*C*). These results suggest that a portion of both the acute and persistent phases is dependent on the CaMKII/GluN2B interaction, as they are attenuated in the KI mice. However, another portion of the persistent reduction in synaptic strength was unexpectedly still present in the GluN2BΔCaMKII KI mice.

A Presynaptic Effect of 20 μM tatCN21 That Is Fully Maintained in GluN2B KI Mice—To determine the mechanism of the persistent reduction, the pre- and postsynaptic components of the effects of 20 μM tatCN21 were further investigated. The presynaptic fiber volley (FV) amplitudes and paired pulse ratios were measured (reflecting the drive of presynaptic release by fiber input and the presynaptic release probability, respectively). It was found that FV amplitudes were significantly reduced by 20 μM tatCN21 treatment and remained persistently decreased after the drug washout ($p < 0.05$; one-way RMANOVA; Fig. 3, *A* and *B*). These results indicate that a significant portion of the reduction in synaptic strength as measured by fEPSPs is due to reduced presynaptic firing. No difference was observed between WT and KI slices regarding the
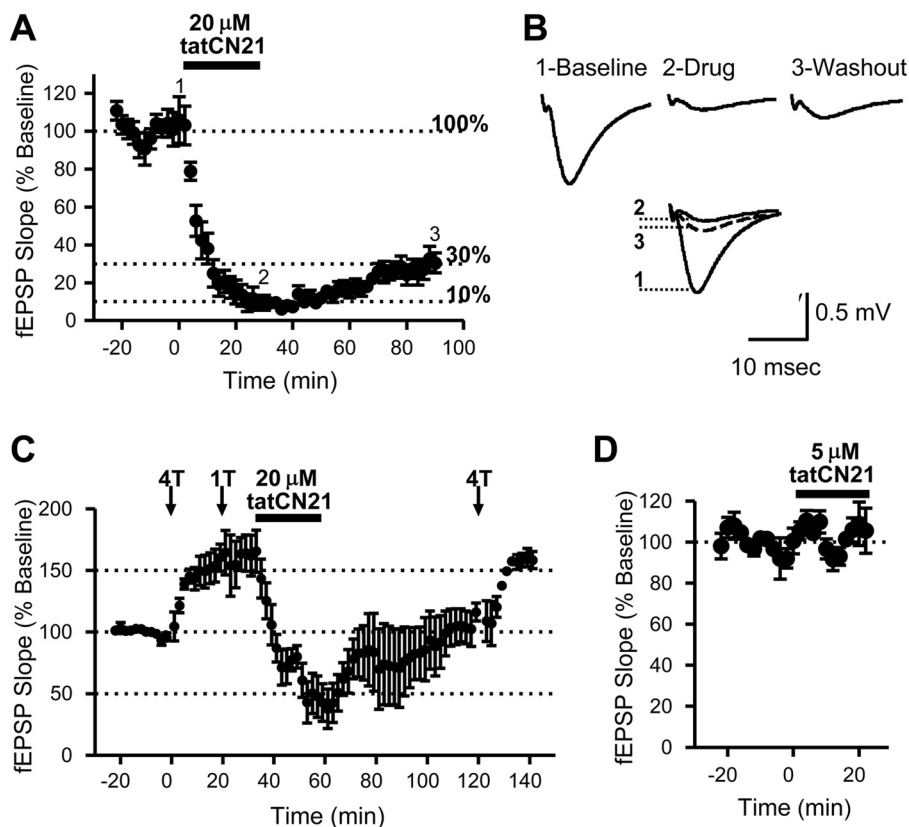
FIGURE 1. **Reduction of synaptic strength by 20 μM tatCN21.** *A*, application of 20 μM tatCN21 for 30 min after at least 20 min of stable baseline reduced basal synaptic strength, which partially recovered during a 60-min washout period in hippocampal recordings from WT slices (*n* = 8). *1, 2,* and *3* indicate the time points shown in *B*. *B*, example fEPSP traces for one experiment from the time course in *A*. After baseline recording (*1*), tatCN21 treatment (*2*) reduced the response size, which partially recovered after washout (*3*). Merged traces are shown below, with the washout response as *dotted line. C*, after at least 20 min of stable baseline, LTP was induced by a four-tetanus protocol (*4T*; 4 × 100 Hz, 1 s) followed by an additional tetanus (*1T*) 20 min later to show saturation. 30 min after the initial four tetani, 20 μM tatCN21 was applied, which reduced potentiated transmission to below the initial baseline value. A 60-min washout led to a partial recovery of the response. Saturation of LTP was reversed, as an additional four tetani after washout reinduced LTP. *D*, the lower concentration of 5 μM tatCN21 (sufficient to block enzymatic activity) was applied after stable baseline and did not reduce basal transmission (*n* = 4), consistent with previous results.

change in FV amplitudes after treatment with 20 μM tatCN21, indicating that this effect is independent of the CaMKII/GluN2B interaction. Using the lower tatCN21 concentration of 5 μM, which is still sufficient to block CaMKII activity and LTP induction (9), there was no reduction in FV amplitudes (Fig. 3*C*), consistent with the lack of effect of 5 μM tatCN21 on the maintenance of synaptic strength. Together, these results indicate that the presynaptic effect of tatCN21 at 20 μM is nonspecific, *i.e.* neither dependent on CaMKII/GluN2B binding nor on CaMKII activity.

Paired pulse ratios were measured in a portion of the WT slices. The facilitation in response amplitudes was significantly higher during 20 μM tatCN21 treatment (*p* < 0.05, one-way ANOVA) but completely returned to baseline levels after washout (Fig. 3*D*). These results indicate that a portion of the initial reduction measured acutely during the drug treatment could be attributed to a decrease in presynaptic release probability, but such an acute reduction does not contribute to the persistent phase after drug washout. Thus, the persistent presynaptic effect of transient 20 μM tatCN21 application is on the drive of release by fiber input, not on release probability. Then, any remaining persistent effect should be postsynaptic.

Postsynaptic Effects of 20 μM tatCN21 Are Largely Absent in KI Mice—To test the postsynaptic contribution to the persistent reduction in synaptic strength that remains after the wash-

out after transient 20 μM tatCN21 treatment, input/output (I/O) curves were generated before and after each experiment (Fig. 4, *A–C*). As the fiber volley amplitude is plotted as the input, these plots eliminate the persistent presynaptic effect of tatCN21. The tatCN21 treatment resulted in a strong persistent reduction of the I/O slope in WT slices (to about half) but only in a minimal reduction in KI slices (by ~10%). Although the reduction in slope was statistically significant for both WT and KI slices (*p* < 0.001 and *p* < 0.01, respectively; two-way ANOVA; Fig. 4*C*), the reduction was much greater in the WT slices (*p* < 0.001; two-way ANOVA; Fig. 4*C*). Thus, at least the major portion of the persistent postsynaptic reduction is no longer present in the KI mice, indicating that most of the postsynaptic effect of 20 μM tatCN21 is indeed mediated by disrupting the CaMKII/GluN2B interaction.

To illustrate an estimate of the postsynaptic component of the tatCN21 effects also over the time course of our experiments, the presynaptic effect on the fiber volleys was subtracted from the total effect on fEPSPs (Fig. 4, *D* and *E*). This manipulation is valid given that the measurements were obtained on the most linear aspect of the I/O relationship and that the fEPSP slopes and fiber volley amplitudes were normalized. It should be noted that the resulting plot does not eliminate the transient decrease in presynaptic release probability during the acute tatCN21 application. However, for the persistent phase after
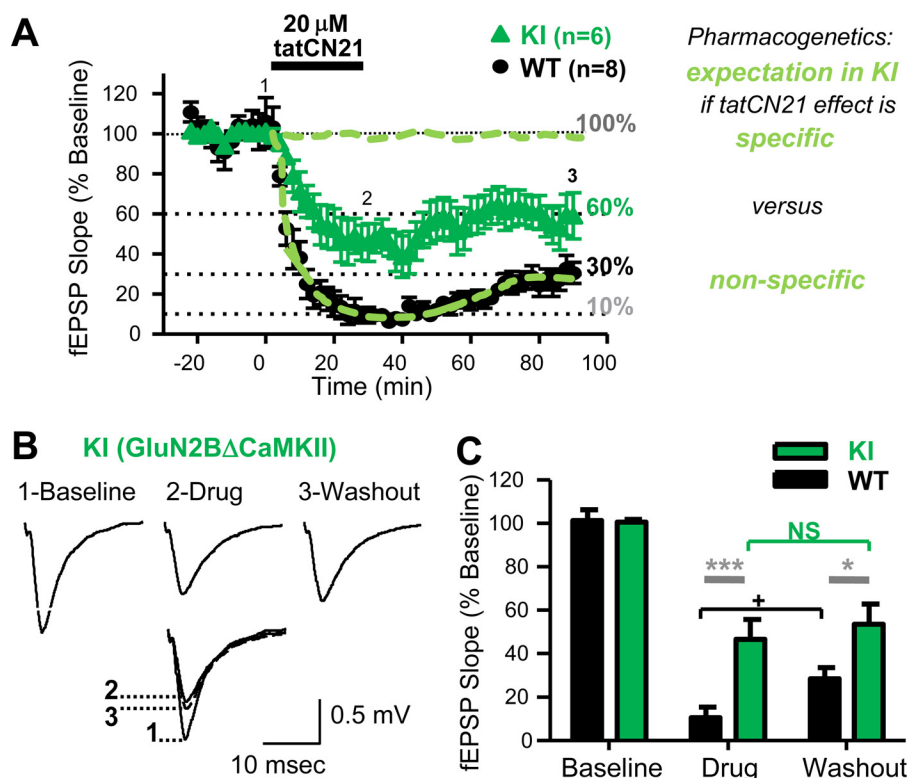
FIGURE 2. **20 μM tatCN21 differentially affects basal transmission of WT and KI mice.** *A*, 20 μM tatCN21 was applied for 30 min after at least 20 min of stable baseline, followed by 60 min of washout in hippocampal recordings. Responses from WT slices (*black circles*, same as in Fig. 1) and from KI slices (*green triangles*) are compared. Reduction of the tatCN21 effect in the KI slice indicates a specific effect by CaMKII/GluN2B disruption in the WT slices; however, the remaining effect must be nonspecific. Results expected based on purely specific or purely nonspecific effects are indicated by *dashed green lines*. *1*, *2*, and *3* indicate the time points shown in *B*. *B*, example traces from KI slices at the time points indicated in *A*, representing baseline (*1*), drug treatment (*2*), and washout (*3*). The traces are shown overlapping below. *C*, quantification of the time course for baseline (−10 to −2 min), drug treatment (22–30 min, and washout (82–90 min) for both WT ($n = 8$) and KI ($n = 6$) recordings. Compared with WT slices, the responses in KI slices were significantly less reduced during both treatment and after washout (two-way ANOVA; *, $p < 0.05$; ***, $p < 0.001$). For the WT, the responses partially recovered after washout (one-way ANOVA; +, $p < 0.05$). For KI, the responses during treatment and after washout did not differ significantly (one-way ANOVA; *NS*, non-significant). In both WT and KI slices, all responses after drug application were significantly reduced compared with baseline (one-way ANOVA; $p < 0.001$).

tatCN21 washout (*i.e.* when the transient effect on release probability is gone (see Fig. 3*D*)), the plot provides a direct reflection of the postsynaptic component. Indeed, the overall persistent postsynaptic effects calculated by this approach are consistent with the postsynaptic effects seen on the I/O slopes. Transient tatCN21 treatment resulted in a strong persistent reduction in WT slices (to about half) but only minimal reduction in KI slices (by ~10%). Together, the results of this study provide the first direct evidence that the CaMKII/GluN2B interaction has an essential function in maintaining synaptic strength (Fig. 5).

Discussion

CaMKII/GluN2B binding is required for the full expression of LTP, as shown by genetic disruption of the interaction (20). This binding has been suggested to be specifically required for the maintenance of synaptic strength, based on pharmacological intervention with the CaMKII inhibitor tatCN21 at 20 μM, a concentrations high enough to disrupt this interaction (23). However, previous studies have indicated that tatCN21 can have additional nonspecific effects at such high concentrations (22, 24). Using a pharmacogenetic approach, the results of this study show that the reduction of synaptic strength by 20 μM tatCN21 has a nonspecific presynaptic component (on fiber volley amplitude), highlighting the limitations of exclusively pharmacological approaches. However, the remaining component of the persistent tatCN21 effect was almost entirely due to disruption of the CaMKII/GluN2B interaction, as this component was almost completely eliminated in the GluN2BΔCaMKII mice that have a mutant GluN2B that is incompetent for CaMKII binding. Our approach combined the specificity of genetic approaches with the temporal resolution of pharmacological approaches and provides the first direct causative evidence that the CaMKII/GluN2B interaction is required for the maintenance of synaptic strength despite also revealing nonspecific effects of tatCN21 at high concentrations.

Notably, at lower tatCN21 concentrations (5 μM) that are not sufficient to disrupt the CaMKII/GluN2B interaction (23), neither the nonspecific effects nor any reduction of synaptic strength were observed even though 5 μM tatCN21 is sufficient to block CaMKII activity and LTP induction (9). Thus, any tatCN21 effects on synaptic strength that were independent of CaMKII/GluN2B disruption were also independent of CaMKII activity inhibition. The molecular targets for the nonspecific presynaptic effects on fiber volleys observed here are unclear, as they likely do not involve the known side effects of increasing the tatCN21 concentration to 20 μM. Increased inhibition of CaMKIV is an unlikely cause, as this kinase has mainly nuclear functions (25). Formation of the unusual CaMKII/polyribosome aggregates is unlikely to have persistent effects, as these
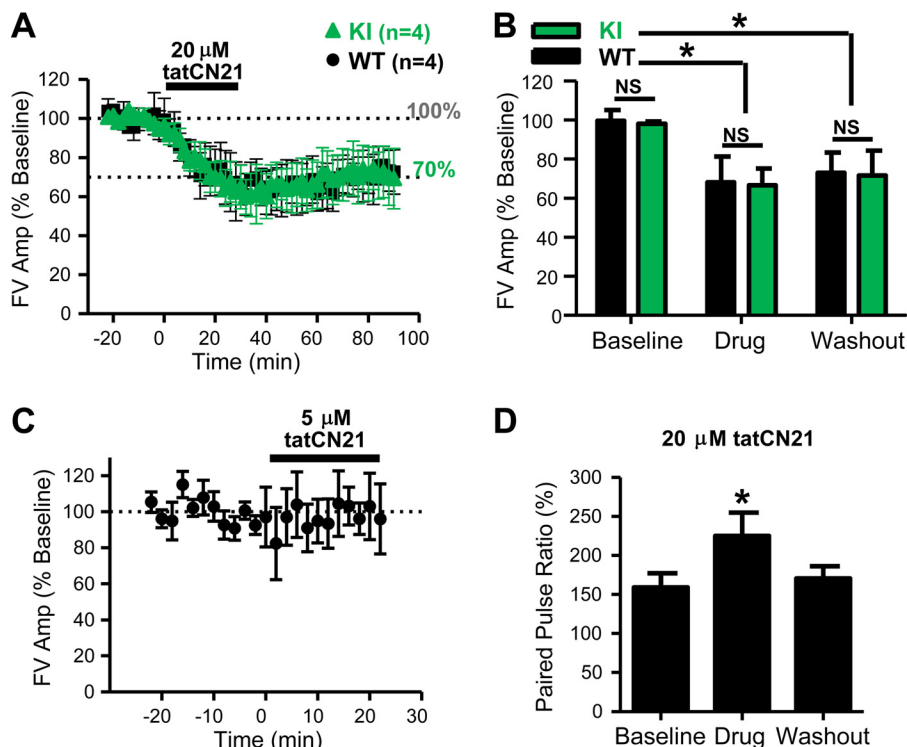
FIGURE 3. **20 μM tatCN21 persistently reduces presynaptic function.** *A*, FV amplitudes (*Amp*) were measured over the same time course as in Fig. 2*A*. In both WT and KI slices, 20 μM tatCN21 reduced FV amplitudes to about 70%, which remained persistently reduced after washout. *B*, quantification of the time course for baseline (−10 to −2 min), drug treatment (22–30 min), and washout (82–90 min). FV amplitudes were significantly reduced compared with baseline during both treatment and washout for both genotypes (two-way ANOVA; *, $p < 0.05$). No differences were seen between WT and KI. *NS*, non-significant. *C*, no reduction in FV amplitudes was observed with 5 μM tatCN21 (*n* = 4), measured over the time course shown in Fig. 1*D*, consistent with previous results that found no change in fEPSC amplitude. *D*, paired pulse ratios were increased during 20 μM tatCN21 treatment in WT mice (compared with baseline; one-way ANOVA; *, $p < 0.05$; *n* = 4) and returned to baseline during washout.

aggregates are completely dissolved after washout of tatCN21 (24). However, our results clearly demonstrate that persistent nonspecific effects were presynaptic, as they were on fiber volley amplitude.

More importantly, however, our pharmacogenetic approach also revealed a significant specific component of the persistent tatCN21 effect that was indeed mediated by CaMKII/GluN2B disruption. Studying this persistent phase of the effect after tatCN21 washout (*i.e.* when tatCN21 no longer acutely blocks CaMKII activity) precludes applying tatCN21 through the patch pipette in whole cell recording to isolate postsynaptic effects from presynaptic effects. Nonetheless, our results clearly show that the nonspecific effects on fiber volleys are presynaptic and also provide strong evidence that the CaMKII/GluN2B-specific persistent effect is instead postsynaptic, as would be expected.

Presynaptic strength is determined by drive of release, release probability, and quantal transmitter content that is released. The only persistent presynaptic effect of 20 μM tatCN21 was on drive of release, reflected by the fiber volleys. Although 20 μM tatCN21 also had an effect on presynaptic release probability, as indicated by increased paired pulse facilitation during drug application, this effect was only transient and completely reversed during the persistent phase after drug washout. A persistent effect of tatCN21 on quantal transmitter content is unlikely, as high-frequency stimulation after tatCN21 washout reinduced LTP to the same level as before. Thus, with no persistent effect on release probability or quantal

content, eliminating the persistent effect on drive of release leaves only postsynaptic effects on synaptic strength. Here the effect on fiber volley amplitude was eliminated by two different approaches (subtraction by math or I/O plots with fiber volleys as input), and both approaches revealed that the remaining postsynaptic effect was largely mediated by CaMKII/GluN2B disruption.

As previously observed in rats (23), transient application of 20 μM tatCN21 persistently reduced not only potentiated but also basal synaptic transmission in mouse hippocampal slices. Importantly, our pharmacogenetic approach showed that a reduction of basal synaptic transmission is indeed caused by the acute disruption of CaMKII/GluN2B binding. But then, why does genetic disruption of this binding in the GluN2BΔCaMKII mice impair only LTP but not basal transmission (20)? Compensatory effects in mutant mice are a common limitation of genetic approaches (26–28). For instance, normal hippocampal LTP in cyclic GMP-dependent kinase II knockout mice is enabled by increased Ca^{2+}-permeable AMPA receptor function (28). In an extreme example, normal physiological LTP is clearly mediated by changes in AMPA receptor currents, yet LTP can still be induced in neurons in which AMPA receptors are completely eliminated, and kainate receptors that are not normally found at these synapses are expressed instead (29). For basal synaptic transmission, compensation should actually be expected through the same mechanisms that mediate homeostatic synaptic scaling. In response to a persistent decrease in synaptic transmission, neurons compensate by
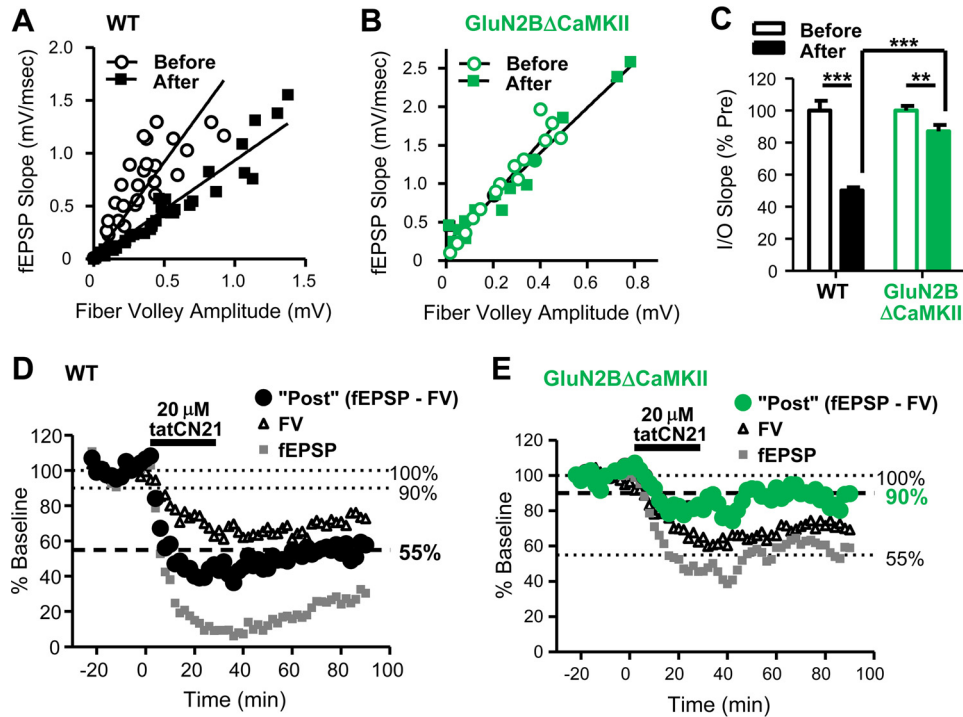
FIGURE 4. **Persistent postsynaptic reduction is nearly absent in GluN2BΔCaMKII KI slices.** *A*, I/O curves for WT slices, generated before and after each treatment with 20 μM tatCN21. The fEPSP slopes are plotted as a function of FV amplitudes. A linear best fit line is shown to compare the I/O relationships, indicating a reduction in postsynaptic function after 20 μM tatCN21 treatment and washout (*n* = 4). *B*, I/O curves as in *A* for GluN2BΔCaMKII mice, revealing a smaller reduction in the I/O relationship by tatCN21 treatment (*n* = 3). *C*, comparing slopes of the best fit lines in *A* and *B*, both normalized to the baseline before tatCN21 treatment. Both WT and KI slices had a significant reduction in slope after treatment, but this reduction was much less in the KI compared with WT mice (two-way ANOVA; **, $p < 0.01$; ***, $p < 0.001$). *D*, calculation of the non-FV contribution to the total effect of 20 μM tatCN21 in WT slices, which approximates the postsynaptic component. Both fEPSP slope data (*gray squares*) and FV amplitude data (*open triangles*) were normalized to percent of baseline. Then the approximate postsynaptic component (*Post*, *black circles*) was calculated by subtracting the normalized FV amplitudes from the normalized fEPSP slopes. The persistent postsynaptic contribution then shows a reduction to about 55% of baseline during the persistent phase in WT slices. *E*, calculation of the non-FV contribution as in *D* but for KI mice. The persistent postsynaptic contribution for KI slices shows only a small reduction during the persistent phase.
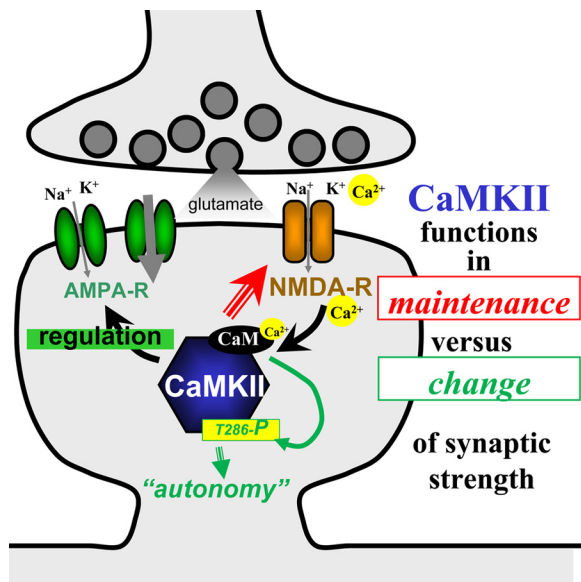

FIGURE 5. **Schematic of an excitatory spine synapse.** CaMKII functions in plasticity *versus* maintenance of synaptic strength are indicated.

increasing the overall strength of their synaptic connections through glutamate receptor trafficking (30). Our results indeed indicate such compensatory effects in CaMKIIΔCaMKII mice as reason for their normal transmission, thus further highlighting the limitations using genetic approaches alone. Fortunately,

compensation after genetic manipulation not only necessitates but actually also enables the pharmacogenetic combination approaches taken here (by enabling testing whether the function that remains after genetic manipulation was retained by lack of an effect or was instead regained by compensation).

The results of this study demonstrate that the role of CaMKII/GluN2B binding in the maintenance of synaptic strength is not LTP-specific. This is consistent with CaMKII being localized to synapses and bound to GluN2B also in the basal synaptic state, with LTP stimuli causing further synaptic CaMKII accumulation and GluN2B binding. However, it has been argued that even this "basal" synaptic state reflects a history of past synaptic stimulation and potentiation (3, 23), and the amount of CaMKII persistently localized to synapses indeed depends on the previous stimulation history (17). Based on this argument, LTP-specificity of a pharmacological intervention would only indicate that the target of such intervention is not maintained long enough after LTP induction or no longer contributes to maintenance of synaptic strength after some time. This would include targets that are required for the transition from early- to late-phase LTP but that are not required for maintaining the late phase-potentiated state itself. Among the few interventions described to specifically reverse LTP without affecting basal synaptic transmission is treatment with the PKC/PKM-ζ pseudosubstrate peptide myrZIP (12–14). However, myrZIP binds promiscuously to other PKC family iso-

forms (31), and its relevant target for LTP reversal is still unknown. In fact, pharmacogenetic evidence made involvement of PKC/PKMζ highly unlikely, as LTP was still completely reversed by myrZIP even in conditional PKC/PKMζ knockout mice (15). The nonspecific effect detected in this study also for high concentrations of tatCN21 further emphasizes the power and importance of such pharmacogenetic approaches. However, in contrast to the myrZIP study, the results presented here additionally show that the CaMKII/GluN2B interaction is one of the targets for the persistent reduction in synaptic strength by transient application of 20 μM tatCN21. Thus, CaMKII/GluN2B binding indeed participates in the maintenance of synaptic strength.

Experimental Procedures

Materials and Animals—All chemicals were from Sigma unless otherwise indicated. The tatCN21 peptide was synthesized by CHI Scientific. The GluN2BΔCaMKII KI mouse has been described previously (20, 32). The mouse carries the mutations L1298Q and R1300A within the CaMKII binding site of GluN2B (20); each mutation individually disrupts CaMKII/GluN2B binding almost completely (18). The mice were backcrossed with C57BL/6 mice from Charles River Laboratories to generate further breeding pairs. All animal use was in accordance with the Institutional Animal Care and Use Committee of the University of Colorado.

Slice Preparation—Mice (21–28 days old) were anesthetized with isoflurane, and their brains were rapidly dissected into ice-cold cutting solution (220 mM sucrose, 3 mM KCl, 1.2 mM NaH_2PO_4, 26 mM $NaHCO_3$, 12 mM $MgSO_4$, 0.2 mM $CaCl_2$, 10 mM glucose, 1.8 mM ascorbate, and 2.15 mM *N*-acetyl cysteine; oxygenated with 95% O_2/5% CO_2). Slices were prepared using a vibratome, cutting 400-μm sagittal sections containing the hippocampus. Throughout slice preparation, the brain remained submerged in cold cutting solution with oxygenation. Area CA3 was removed from each section individually with a scalpel. Slices recovered for at least 90 min in ACSF (126 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgSO_4$, 26 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 10 mM glucose, and 1.8 mM ascorbate) with 2.15 mM *N*-acetyl cysteine, oxygenated and warmed to 32 °C.

Electrophysiology—Hippocampal recordings were collected as described previously (1, 9). Briefly, slices were placed in a recording chamber and submerged in recirculating ACSF at 31–32 °C. Recording electrodes (filled with ACSF) were placed in the stratum radiatum. Responses were stimulated in the Schaffer-Collateral pathway every 20 s by a bipolar tungsten wire electrode. At the beginning (and the end) of each experiment, input/output curves of field excitatory postsynaptic potential (fEPSP) amplitudes were generated. Then, test stimuli eliciting ~60–80% of the maximal response were used for experiments testing effects on basal transmission. For potentiation experiments, ~30–40% was used instead. All healthy slices with fEPSP amplitudes greater than 0.5 mV and FV amplitudes less than half the size of fEPSP amplitudes were included; all other slices were excluded from the analysis. Also excluded were any data that contained contaminating population spikes. The comparison of wild-type and GluN2BΔCaMKII included both littermates from heterozy-

gous breeding and non-littermates from homozygous breeding. For tatCN21 treatment, the peptide was added to the recirculating fluid for 30 min, followed by washout, induced by switching from recirculation to flow-through. Paired pulse ratios were measured with an interpulse interval of 50 ms during baseline, treatment, and washout over 6 responses (2 min) each. Slopes of fEPSPs and FV amplitudes were measured using WinLTP software. Potentiation was induced with four tetani (100 Hz over 1 s, intertrain interval of 20 s), with an additional tetanus to demonstrate saturation.

Analysis and Statistics—Statistical results were generated as indicated using GraphPad Prism. For all experiments, *n* represents the number of slices. To analyze time courses, fEPSP slopes and FV amplitudes were averaged over 10 min (*i.e.* 30 responses) before drug treatment (−10 to −2 min), during drug treatment (22–30 min), and after washout (82–90 min) for each individual slice recording. These averages were then compared using a repeated measures analysis of variance (RMANOVA). To analyze input/output curves, best-fit lines with fixed intercept were generated, and the resulting slopes were compared using ANOVA. For ANOVA analysis, post-hoc Bonferroni analysis was used when necessary.

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