The role of hippocampal CaMKII in resilience to trauma-related psychopathology

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ARTICLE INFO

Keywords:
PTSD
CaMKII
Stress resilience
Dentate gyrus
Long-term potentiation

ABSTRACT

Traumatic stress exposure can form persistent trauma-related memories. However, only a minority of individuals develop post-traumatic stress disorder (PTSD) symptoms upon exposure. We employed a rat model of PTSD, which enables differentiating between exposed-affected and exposed-unaffected individuals. Two weeks after the end of exposure, male rats were tested behaviorally, following an exposure to a trauma reminder, identifying them as trauma ‘affected’ or ‘unaffected.’ In light of the established role of hippocampal synaptic plasticity in stress and the essential role of Ca2+/calmodulin-dependent protein kinase II (CaMKII) in hippocampal synaptic plasticity, we pharmacologically inhibited CaMKII or knocked-down (kd) αCaMKII (in two separate experiments) in the dorsal dentate gyrus of the hippocampus (dDG) following exposure to the same trauma paradigm. Both manipulations brought down the prevalence of ‘affected’ individuals in the trauma-exposed population. A day after the last behavioral test, long-term potentiation (LTP) was examined in the dDG as a measure of synaptic plasticity. Trauma exposure reduced the ability to induce LTP, whereas, contrary to expectation, αCaMKII-kd reversed this effect. Further examination revealed that reducing αCaMKII expression enables the formation of αCaMKII-independent LTP, which may enable increased resilience in the face of a traumatic experience. The current findings further emphasize the pivotal role dDG has in stress resilience.

1. Introduction

Post-traumatic stress disorder, or PTSD, is recognized as a major health challenge, but there are only partially effective treatments for the disorder. Often, PTSD co-occurs with mood and anxiety-related disorders and is associated with a severe disability and medical illness (Nestler and Hyman, 2010; Shalev et al., 2017). One significant obstacle hindering clinical solutions to PTSD resides in the individual variability that exists in susceptibility to trauma (Richter-Levin et al., 2019a; Richter-Levin and Sandi, 2021). Epidemiological data demonstrated that even after experiencing severe, traumatic stress, only about 7% of the exposed individuals would display symptoms of PTSD (Goswami et al., 2013; Kessler et al., 2005a; Olaya et al., 2015). When exploring risk factors that may explain this individual variability, childhood adversity in humans (McLaughlin et al., 2017) and juvenile stress in animal models (Albrecht et al., 2017a; Horovitz et al., 2014; Ritov et al., 2017) were found to significantly exacerbate the impact of adult stress on activity and anxiety-like behaviors.

PTSD has been repeatedly documented to involve reduced hippocampal functioning (Chen and Etkin, 2013) and volume (Levy-Gigi et al., 2015; Woon et al., 2010). Experimental evidence indicates that the dorsal hippocampus, which corresponds to the human posterior hippocampus, is specifically involved in the formation of stable ‘declarative’ memory (Squire, 1992) and in encoding cognitive and spatial information (Bannerman et al., 2014; Eichenbaum et al., 1999; Fanselow and Dong, 2010a). The ventral or temporal pole of the hippocampus, which corresponds to the anterior hippocampus in humans, modulates emotional and affective processes such as stress responses and fear...
behavior (Fanselow and Dong, 2010b; Henke, 1990; Herman et al., 2005). Nevertheless, pharmacological and genetic manipulation derived studies have demonstrated a role for the dorsal hippocampus, and particularly the dorsal dentate gyrus (dDG), in mediating stress effects on hippocampal functioning (Fa et al., 2014) Altered expression of GABAergic factors (Albrecht et al., 2016; Hadad-Opahir et al., 2014; Tripathi et al., 2021) and altered local circuit activity within the dDG (Ardi et al., 2014; Yarom et al., 2008), have been implicated in response to stress and stress resilience. In particular, the hippocampal DG was shown to respond differently to various stressors, enabling it to integrate both emotional and cognitive aspects of an experience and share that data with the hippocampus proper, as well as with a network of brain regions such as the amygdala and prefrontal cortex (Albrecht et al., 2017a; Fa et al., 2014).

Long-term potentiation (LTP) is suggested to serve memory formation (Dringenberg, 2020; Nicoll, 2017). Stress is well known to modulate hippocampal LTP as well as hippocampus-dependent learning and memory. Alterations of plasticity in the hippocampus, amygdala, and medial prefrontal cortex were found to play a pivotal role in stress vulnerability, pathology, and stress resilience (Richter-Levin et al., 2019b; Schmidt et al., 2013). Exposure to stress differently alters neural plasticity in these regions. For example, while exposure to stress was constantly shown to impair LTP in the CA1 field of the hippocampus, the plasticity aberrations induced by stress in the DG are much less consistent (Bergado et al., 2011; Foy et al., 1987; Kim and Diamond, 2002; Vouimba et al., 2020).

One leading player in the induction and maintenance of LTP in the hippocampus is Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) (Bayer and Schulman, 2019; Pettit et al., 1994; Thomas et al., 1994). The mammalian CaMKII is a dodecameric holozyme containing 12 sub-units. Four genes encode CaMKII-α, β, γ, and δ that exhibit alternative splicing in their variable linker domain. Expression level of CaMKII is much higher in the dorsal hippocampus than the ventral hippocampus (von Ziegler et al., 2022). The α and β isoforms are predominant in the hippocampus (up to 2% of total protein) (Coultrap and Bayer, 2012; Lisman et al., 2012). The α isoform is much more abundant than the β isoform in the forebrain in a ratio of 3:1, and the localization of αCaMK-II is mostly in glutamatergic neurons (Miller and Kennedy, 1985; Mohanan et al., 2022).

CaMKII is vital for hippocampal plasticity. NMDA-dependent LTP in CA1 requires CAMKIIndependent LTP to a major contributor to plasticity (Lu et al., 2006). Hippocampal plasticity serves memory formation (Lisman, 1994; Lisman et al., 2012; Sanhueza and Lisman, 2013), and altered memory and altered hippocampus dependent memory are assumed to contribute to PTSD-like symptoms (Richter-Levin et al., 2019c). Glococorticoid hormones (GCs), acting through hippocampal mineralocorticoid receptors (MRs), were found to regulate the Camk2a gene (Mifsud et al., 2021). It can thus be expected that altering CaMKII functionality in the hippocampus will impact the formation of hippocampus dependent traumatic memory and by that impact PTSD-like symptoms (Cho et al., 2007; Niewoehner et al., 2007; Yin et al., 2017a). Several studies have examined the potential role of the αCaMKII in anxiety-related behaviors. Overall, the findings indicate that manipulating the expression or functioning of αCaMKII affects anxiety symptoms, though many inconsistencies still need further investigation (Chen et al., 1994, 2012; Hasegawa et al., 2009; Matsumoto et al., 2013; Yamasaki et al., 2008). We have previously found that the DG has an important role in defining the outcome of exposure to trauma (Fa et al., 2014). Having both CAMKII-dependent plasticity and other types of LTP, with the possibility of a competition between them may be one way to contribute to defining trauma outcome. We here thus focus on examining the impact of manipulating αCaMKII functionality in the DG.

Considering the impaired memory, learning and neural plasticity in the hippocampus of PTSD subjects (Geuze et al., 2009) and the significant role CaMKII plays in neuroplasticity, we hypothesize that αCaMKII may induce pathological plasticity in the hippocampus resulting in the distortion of memories and maladaptive enhancement of emotional events. The main goal of this study is to examine whether alterations in CaMKII expression will influence the percentage of PTSD-affected individuals. Specifically, we focus on reducing αCaMKII expression in the dDG as this sub-region of the hippocampus has been implicated in defining stress vulnerability or resilience (Albrecht et al., 2017a, 2021a; Fa et al., 2014; Ritov et al., 2016; Tripathi et al., 2021). Furthermore, in order to relate to individual variability in response to trauma (Richter-Levin and Sandi, 2021), we employed our recently developed behavioral profiling approach (Ardi et al., 2016; Cohen et al., 2012; Cohen and Zohar, 2004; Regev-Tsur et al., 2020), which enables dissociating trauma-exposed-affected from trauma exposed-unaffected individuals.

2. Materials and methods

2.1. Experimental groups and design

Experiment 1. The impact of combined juvenile stress and underwater trauma (JS-UWT) on the prevalence of ‘affected’ animals.

In order to assess the prevalence of ‘affected’ animals, acclimated rats were randomly assigned to two groups: control (CTR; n = 18) and juvenile stress + underwater trauma (JS-UWT) (n = 24).

Experiment 2. The impact of pharmacological inhibition of CaMKII on the percentage of affected individuals.

In order to evaluate the impact of pharmacological inhibition of CaMKII in the dDG of the hippocampus on the percentage of affected individuals, another set of rats were randomly assigned to three groups: (a) control-saline (CTR-SAL; n = 12), (b) juvenile stress + underwater trauma-saline (JS-UWT + SAL) (n = 18) and (c) juvenile stress + underwater trauma + KN93 inhibitor (JS-UWT + KN93; n = 22).

Experiment 3. The impact of reduced expression of CaMKII on the percentage of affected individuals.

Rats were injected with an AAV-αCaMKII-kd viral construct (αCaMKIIv) to selectively reduce αCaMKII levels in the dDG. Rats were randomly assigned to three groups: control (CTR; n = 12), juvenile stress + underwater trauma – control virus (JS-UWT + CtrV; n = 17), juvenile stress + underwater trauma– αCaMKII Knockdown (JS-UWT+αCaMK II V; n = 18).

Experiment 4. - The impact of reduced expression of CaMKII on DG plasticity.

A separate set of animals were injected with αCaMKIIv in dDG to examine the effect of CaMKII-kd on LTP induction in vivo without the potential confounding effects of behavioral tests. Rats were randomly assigned to three groups: Naive control (CTR; n = 14), Control virus (Ctr V; n = 14), and αCaMKII Knockdown (αCaMK V; n = 16). These animals were not subjected to any stress protocol or behavior tests.

2.2. Animals

Male Sprague-Dawley rats weighing 30–50 gm (Envigo, Jerusalem, Israel) were grouped (four per cage) upon arrival [postnatal day (PND) 22] and habituated for five days (22 ± 2 °C). They had regular access to food pellets and water ad libitum throughout the procedure. Animals were housed on a 12:12-h, light–dark cycle (lights on 07–19 h). All experiments were approved by the University of Haifa Animal Care and Use Committee and performed according to the NIH Guidelines.

2.3. Stress protocols

2.3.1. Juvenile stress (JS.)

The juvenile stress protocol was implemented, as previously described (Ardi et al., 2016; Horovitz et al., 2014; Regev-Tsur et al.,
Three different stressors, one each day, were applied for three consecutive days (PND 27–29) under full light conditions. Day 1: Rats received 10 min of forced swim stress; Day 2: Elevated platform - Rats were placed on an elevated platform for 3 × 30 min (1 h of inter-set interval in the home cage); Day 3: rats received 2 h of restraint stress.

2.3.2. Adulthood stress - underwater trauma (UWT)

The UWT stress protocol was followed as previously described (Ardi et al., 2016; Regev-Tsur et al., 2020). Young adult rats were habituated in a standard cage with a plastic lid for four days (PND 60–63), 2 min per day. On the fourth day (PND 63), following the 2 min habituation trial, all rats were exposed to vanilla odor for 30 s (3 puffs, 10 s intervals) inside the cage. Immediately after the odor exposure, all rats except the controls were immersed in a water-filled plastic tank. After a few seconds of free swimming, they were restrained underwater for 45 s. Control rats were kept in their home cages following odor exposure without UWT.

2.4. Behavioral assessments

Two weeks after the UWT, rats were behaviorally assessed in the open field (OF) and the elevated plus-maze (EPM), as described below. Animal behavior was recorded and then analyzed using the Etho-Vision XT8 video tracking system (Noldus, Wageningen, Netherlands). Experimenter were properly blinded to treatment conditions.

2.4.1. Trauma reminder

Odor re-exposure was employed as a reminder cue (Ardi et al., 2016; Regev-Tsur et al., 2020). Before each behavioral test, animals were re-exposed for 30 s to the same vanilla odor used prior to the UWT exposure. Rats were subjected to the behavioral tests immediately after.

2.4.2. Open field (OF) test

Locomotor activity and anxiety-like behavior were tested at PND 77 (Ardi et al., 2016; Tripathi et al., 2021). Following trauma reminder, rats were placed in the corner of the OF (under dim light illumination) and were left to explore the arena for 5 min. The anxiety index was expressed as a percentage.

\[
\text{Anxiety index} = \frac{\text{Distance Covered or Time explored in the centre}}{\text{Total distance or Total time}} \times 100
\]

2.4.3. Elevated plus maze test (EPM)

EPM was carried out 24 h after the OF test (PND 78) as previously described (Ardi et al., 2016; Tripathi et al., 2021). Briefly, rats were placed in the center to explore the maze for 5 min. The anxiety index was expressed as a percentage.

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\text{Anxiety index} = \frac{\text{Distance Covered or Time explored in the open arm}}{\text{Total distance or Total time in open and closed arm}} \times 100
\]

2.4.4. Behavioral profiling with an algorithm

To dissociate between trauma “affected” and “unaffected subjects,” we implemented the “behavioral profiling” approach, which refers to the performance of control, a non-exposed group as characterizing normal behavior, and assessed deviation from the “norm” (Ardi et al., 2016). Classification criteria were defined according to the control group variability in performance for each behavioral parameter. Based on validated previous experience (Ardi et al., 2016; Regev-Tsur et al., 2020; Ritov et al., 2016; Tripathi et al., 2021), we here developed an algorithm (Supplementary Fig. S1) which screened fifteen different behavioral variables from the OF and EPM, which represent activity and anxiety-like behaviors (Supplementary Table S1) (For a detailed description see Supplementary details).

2.5. Stereotoxic surgery

Stereotoxic surgery PND 44–45(Tripathi et al., 2021) was performed bilaterally in the dDG relative to bregma (AP = −3.7; Lateral = ± 2.2; Ventral = −3.6 mm) according to Paxinos and Watson (Paxinos and Watson, n.d.). For a detailed description, see Supplementary details.

2.5.1. Cannula implantation and KN 93 drug microinjection

During the surgery procedure (PND 44–45), 22-gauge stainless steel guide cannulae were implanted bilaterally, 2 mm above the dDG. The cannulae and two anchoring screws were secured by dental cement. Stylets were inserted into the cannulas and removed only for drug infusion. Following two weeks of JS (PND 60), rats were divided randomly into two groups to be exposed to the UWT. On PND 63, one group of JS-UWT rats received specific CaMKII inhibitor KN-93 (N-[2-[N-(4-Chlorocinnamyl)-N-methylaminomethyl] phenyl]-N-(2 hydroxyethyl)-4-methoxybenzenesulfonamide phosphate salt (#K1385, ≥98% HPLC, Sigma-Aldrich, USA) 15 min before UWT. KN-93 was applied locally in the dDG at a dose of 5 μg/0.5 μl per hemisphere, dissolved in saline (0.09%) before injection. In each hemisphere, the other group received 0.5 μl of saline (0.09%). KN-93 or saline was microinjected through a 27-gauge injection needle (protruded 2 mm beyond the tip of the cannula) connected with polyethylene tubing to a 2 μl Hamilton syringe in the dDG of JS-UWT grouped rats. The microinjection of 0.5 μl was performed over a 60 s for each side in each experiment. Cannula placement was verified histologically, and only those animals with the correct location in the DG area were included in the final analysis.

2.5.2. Lentivirus production and validation

The design and construction of pAAV-CMV-αCaMKII-P2A-EGFP (AAV- αCaMKII, inducible), pAAV-U6-αCaMK2.shRNA-CMV-EGFP (AAV-αCaMKII-kd) and AAV-CMV-EGFP-2A-MCS-3FLAG (AAV-control) virus vectors (1.6E × 13 vector genomes/ml) were based on (Gray, 2011) and packaged by Obio Technology Co. Ltd. (Shanghai, China). The reduction of αCaMKII in vitro was validated and quantified by western blotting. Furthermore, in vivo validation was performed by Western blot analysis following αCaMKII knockdown in the rat dDG. (For a detailed description, see Supplementary details).

2.5.3. Viral injection

On PND 44–45, rats received bilateral microinjections of either AAV-control or AAV-αCaMKII-kd virus vector in the dDG. 1 μl of viral vector suspension was injected in each hemisphere (0.15 μl/min) through a 10 μl Hamilton syringe (30G) connected to a motorized nanoinjector (Stereotoxic Injector, Stoelting, Wood Dale, USA) (Saha et al., 2018; Tripathi et al., 2021).

2.6. In vivo electrophysiology and induction of long-term potentiation (LTP)

The electrophysiology procedure was conducted as described before (Ardi et al., 2014; Tripathi et al., 2021) in the dentate gyrus –perforant pathway (DG-PP). Two TBS stimulation protocols were used - a regular TBS protocol and a strong TBS protocol. Regular protocol: a single set of 10 trains, each of 10 pulses, administered at 100 Hz with 200 ms inter-train interval. Strong protocol: three sets of 10 trains, each of 10 pulses, administrated at 100 Hz with 200 ms inter-train interval and 1 min inter-set interval. LTP was recorded for 60 min after TBS and measured as the difference in EPSP slope before and 60 min after TBS (For a detailed description, see Supplementary details).

2.7. Brain removal and histology

Brain samples were collected immediately after the last behavioral experiments or four weeks after the virus injection (Paxinos and Watson, n.d.). (For a detailed description, see Supplementary details)
2.8. Western blot assay

For a detailed description, see Supplementary details.

2.9. Statistical analysis

Data were analyzed using the IBM SPSS (21) Statistics software (IBM, Armonk, NY, USA). All behavioral, electrophysiological, and immunoblot results were analyzed using independent sample t-test, one-way ANOVA, and repeated measures ANOVA followed by post-hoc Bonferroni, as appropriate. For behavioral profiling, Pearson’s chi-square test was used. Appropriate Greenhouse-Geisser or Huynh-Feldt corrections for sphericity issues were applied when necessary. All values are presented as mean ± SEM.

3. Results

3.1. Combined juvenile stress and UWT (JS-UWT) increase the prevalence of ‘affected’ animals

We have earlier shown that pre-exposure to JS exacerbates the effects of exposure to trauma in adulthood, increasing the percentage of affected individuals (Albrecht et al., 2017a; Ardi et al., 2016; Ritov et al., 2017). Thus, we applied a PTSD paradigm consisting of JS and UWT in adulthood (Experimental paradigm 1, Fig. 1A). The behavioral responses of individual animals were tested in the OF and EPM following re-exposure to the UWT-associated odor cue (Ardi et al., 2016). Looking first at the group averages of the behavioral tests revealed that JS-UWT animals showed a significant reduction in the total distance covered in the OF ($t = 3.75$, $p < 0.001$) (Fig. 1B–C) and in the EPM ($t = 4.46$, $p < 0.001$), compared to that of the CTR group. Additionally, there was an enhanced anxiety-like behavior indicated by the reduction in the anxiety index both in the OF ($t = 6.90$, $p < 0.001$) and the EPM ($t = 4.73$, $p < 0.001$).
0.001). Since some individuals are more susceptible to developing aferective symptomatology after a traumatic experience, comparing the different groups’ means would not reveal differences at an individual level between subjects who coped with trauma and those who developed pathology. Hence, we applied the behavioral profiling approach (ardi et al., 2016; regev-tsur et al., 2020) to identify vulnerable and resilient individuals during the exploration tests. Behavior profiling analysis, which employs a dedicated algorithm, including fifteen behavioral parameters extracted from the OF and the EPM tests, identified affected and unaffected individuals. Comparisons between the groups using Pearson \( \chi^2 \) analysis revealed that the proportion of affected rats in the JS-UWT group was significantly higher (83.0%) compared to that of the control group (5.0%), \( (x^2 = 123.5, p < 0.001) \) (fig. 1D).

3.2. Reducing CaMKII functionality in the dDG of trauma-exposed animals decreases the prevalence of "affected" individuals

Next, we examined whether alterations in CaMKII functionality, specifically in the dDG, would affect the percentage of affected individuals. For this purpose, we implemented two different intervention strategies: pharmacological inhibition of CaMKII by KN93 and a local viral-vector-mediated knockdown of aCaMKII expression (Experiments 2 and 3).

3.2.1. Inhibition of dDG CaMKII by KN93 reduces the prevalence of "affected" individuals

Experimental paradigm 2 is depicted in fig. 1E. One-way ANOVA revealed a significant effect for groups in total distance travelled in the OF (\( F_{(2, 49)} = 5.627, p = 0.006 \)) and EPM (\( F_{(2, 49)} = 10.564, p < 0.001 \)), as well as in the anxiety index measured in the OF (\( F_{(2, 49)} = 9.099, p < 0.001 \)) and EPM (\( F_{(2, 49)} = 40.83, p < 0.001 \)) (fig. 1F-G). As expected, exposure to JS-UWT increased anxiety levels both in the OF and EPM, as denoted by the reduction in the total distance covered in the OF and EPM compared to that of the saline-injected, non-trauma-exposed group (Post-hoc Bonferroni \( p = 0.017 \) and \( p < 0.001 \), respectively). A similar effect was found for the anxiety index in EPM (Post-hoc Bonferroni \( p < 0.001 \)). The anxious-like behavior in the EPM was counteracted by the injection of the CaMKII inhibitor KN9-3 before subjecting the rats to adulthood trauma. However, it did not significantly affect the anxiety index in the OF. KN-93 significantly increased the total distance covered in the OF vs. the saline-injected JS-UWT group (Post-hoc Bonferroni \( p = 0.019 \)), but a significant change was not observed in the relative time spent in the center of the arena. The results in the EPM were more prominent than the OF, both for total distance and relative time spent in the open arms (Post-hoc Bonferroni \( p = 0.0013 \) and \( p < 0.001 \), respectively), suggesting that local inhibition of CaMKII in the dDG of adult rats is enough to attenuate their anxiety-like behavior. In line with the above, behavioral profiling analysis revealed a significantly higher proportion of affected rats in the JS + UWT group (76.0%) compared to that of the control group (\( x^2 = 98.507, p < 0.001 \)). KN93 to the dDG before UWT significantly increased the resilience of the JS-UWT exposed rats, raising the proportion of unaffected individuals from 24.0% to 91.0% (\( x^2 = 69.31, p < 0.001 \)) compared to JS-UWT-exposed animals injected with saline (fig. 1H).

3.2.2. The impact of the knockdown of aCaMKII in the dDG

3.2.2.1. Verification of the efficacy of viral knockdown of CaMKII expression in vitro and in vivo. To verify the viral vector’s efficacy and specificity, we assessed viral transfection efficiency and aCaMKII knockdown efficacy in vitro (fig. 2A-C). Since there was no aCaMKII, we transfected AAV-aCaMKII (inducible, A0), and AAV-aCaMKII-kd (knockdown, A1, A2 and A3) vectors in 293T cell line. Subsequent western analysis revealed a robust knockdown of aCaMKII (A0+A1). Results showed that AAV-aCaMKII-kd (A1) had the best knockdown effect compared to the control (\( p < 0.001 \)) (fig. 2C). We further tested the efficacy of the AAV-aCaMKII-kd vector in vivo within dDG. Brain samples from dorsal DG of both hemispheres were collected (Paxinos and Watson, n.d.) four weeks after the virus injection. Only animals with correct injection sites on both hemispheres were included in the analysis, as ascertained by GFP expression (fig. 2D). Local injection of AAV-aCaMKII-kd into the dDG resulted in ~45% knockdown of endogenous aCaMKII expression of control level (\( t = 3.606, df = 13, p = 0.003 \)) (fig. 2E).

3.2.2.2. Knockdown of aCaMKII in the dDG reduces the prevalence of "affected" individuals. Animals were injected with the AAV-aCaMKII-kd virus vector, into the dDG two weeks after JS (PND 44–46). They were later subjected to UWT in adulthood (PND 63) and tested in the OF and EPM two weeks later (PND 77–78; fig. 3A). In the OF, no group effect was evident in the arena path length, although an apparent effect in the total distance was seen in the EPM following exposure to JS-UWT (One-way ANOVA F (2,44) = 7.746 p = 0.001). In line with the previous findings, a significant effect for groups was found for the anxiety index measured both in the OF (\( F_{(2,44)} = 9.303, p < 0.001 \)) and EPM (One-way ANOVA (\( F_{(2,44)} = 12.50, p < 0.001 \)) (fig. 3B-C). As expected, exposure to trauma increased anxiety levels as marked by the reduction in the total distance covered in the EPM (Post-hoc Bonferroni \( p = 0.001 \)) and anxiety index in both OF and EPM (Post-hoc Bonferroni \( p < 0.001 \)).
< 0.001, respectively), compared to the control group. The post-hoc test did not find any significant changes after the knockdown of αCaMKII in the total distance (p = 0.61) and anxiety index (p = 0.12) in the OF. In contrast, in the EPM, a prominent significant effect for total distance and anxiety index was observed, compared to that of the JS-UWT group injected with a control virus (Post-hoc Bonferroni P = 0.042 and p < 0.001, respectively). In line with the above, behavioral profiling analysis revealed a significantly higher proportion of affected rats in the JS-UWT+αCaMKII V group (84.0%) compared to the CTR population, whereas in JS-UWT+αCaMKII V group the affected population is significantly less compared to JS-UWT+CtrV group. Values are the % affected and unaffected animals in each group. ***p < 0.001, ###p < 0.001. (E) Effect of αCaMKII knockdown on synaptic plasticity in the DG of the hippocampus. (i) Evoked field potential response recorded in the DG of CTR (n = 8), JS-UWT (n = 8), JS-UWT+CtrV (n = 10), and JS-UWT+αCaMKII V (n = 10); and (ii) no significant differences found in the input-output curve. (F) A significant DG field potentiation was recorded after TBS in CTR group and JS-UWT+αCaMKII V, while TBS failed to induce potentiation in JS-UWT and JS-UWT+CtrV group (i), changes in average percentage of LTP after TBS (ii). LTP was assessed upon theta-burst stimulation (TBS) to the perforant path-DG pathway, and recording in the dDG. No significant difference in baseline recording was found prior to TBS. Average percentage of LTP (right), The results are the Mean ± SEM. ***p < 0.001, **p < 0.01.

3.3. Selective αCaMKII knockdown enabled LTP in dDG in exposed animals

Twenty-four hours after the last behavioral test, LTP was examined in the dDG in all four groups to measure synaptic plasticity. There was no significant difference in the input-output curve between groups, indicating no baseline difference in excitability (Fig. 3E). No significant difference in baseline recording was found prior to TBS. Strong theta-burst stimulation (TBS) was applied to the PP-DG pathway, and its impact was recorded in the dDG (Fig. 3E). After TBS, mixed model repeated measures of ANOVA showed a significant difference between groups (group effect F (3, 28) = 15.44, p < 0.001; time effect; F (2,199, 60.442) = 7.22, p < 0.001; time X group; F (6.476, 60.442) = 3.44, p < 0.001). JS-UWT reduced the ability to induce LTP (Fig. 3F). Selective knockdown of αCaMKII reversed this effect in the JS-UWT, and the potentiation level was similar to the control. Post-hoc comparisons between the four groups revealed that JS-UWT and JS-UWT+CtrV groups were significantly different from CTR (Bonferroni, p = 0.002 and p < 0.001 respectively) and JS-UWT+αCaMKII V (Bonferroni, p = 0.002 and p < 0.001 respectively) (Fig. 3F). No significant difference was found between CTR and JS-UWT+αCaMKII V group.

3.4. αCaMKII knockdown inhibits LTP induced by regular TBS protocol but not by strong TBS protocol

In the findings above, there was a correlation between the behavioral and LTP results, such that JS-UWT led to pathological symptoms and
impaired LTP while knocking down dDG αCaMKII reduced pathological symptoms and enabled normal levels of LTP. While this correlation seems to suggest a link between the two, the LTP results do not agree with previous reports suggesting that hippocampal LTP is dependent on αCaMKII activation (Rossetti et al., 2018; Soderling, 2000; Wayman et al., 2008). Thus, recovery of the ability to induce LTP by reducing the expression of αCaMKII requires consideration. We set out to examine the possibility that the ability to induce LTP in αCaMKII-kd animals was because we applied a Strong TBS protocol. For that, we compared the effect of αCaMKII-kd on LTP induced by either a Strong or a regular TBS protocol in animals that were not exposed to any behavioral protocol (Fig. 4A). The results support our assumption showing that αCaMKII-kd impaired LTP following the regular TBS protocol. No potentiation was evident after 60 min, and levels did not differ from those of the TBS baseline. There was no significant difference in the input-output curve between groups and baseline recording prior to TBS (Fig. 4B). A repeated measure of ANOVA shows a significant group effect $F(2,22) = 28.09, p < 0.001$; time effect $F(1.802, 39.66) = 4.37, p < 0.001$; but no time x group effect $F(3.605, 39.66) = 2.06, p > 0.05$ (Fig. 4C). Post-hoc comparisons revealed that the αCaMKII V group significantly differed from the CTR and Ctr V groups ($p < 0.001$). Moreover, the comparison of averaged potentiation across time points after induction also showed a significant difference (one-way ANOVA, $F(2,22) = 21.09, p < 0.001$; post-hoc Bonferroni CTR vs. αCaMKII V, $p < 0.001$; and Ctr V vs. αCaMKII V, $p < 0.001$) (Fig. 4C). In contrast, LTP induced by Strong TBS was maintained in all three groups (CTR, $n = 6$; Ctr V, $n = 6$; and αCaMKII V, $n = 7$) (Fig. 4D–E). A repeated measure of ANOVA revealed no significant group effect $F(2,16) = 2.530, p > 0.05$; time effect $F(1.996, 27.94) = 1.146, p > 0.05$; time x group effect $F(3.991, 27.94) = 0.687, p > 0.05$). Furthermore, the comparison of averaged potentiation across time points after induction also showed no significant difference between groups.

4. Discussion

PTSD has been repeatedly documented to involve reduced hippocampal functioning and decreased hippocampal volume (O’Doherty et al., 2015), with a suggested emphasis on the DG (Fa et al., 2014; Qin & 2014; Qin et al., 2015).
Studies focusing on the dorsal hippocampus have found an impact of stress in that region, particularly relating to resilience, expression of GABAergic factors and local circuit activity in the DG (Albrecht et al., 2021a; Tripathi et al., 2021).

The involvement of CAMKII in hippocampal plasticity and the role of pathological hippocampal plasticity in PTSD raises the possibility of a role of CAMKII in stress and trauma. However, the nature of its involvement is not explicit. In an αCaMKII-GFP transgenic mouse line, the αCaMKII-GFP was found to be mostly expressed in the DG region and CA1/CA3. About 70% of granule and pyramidal neurons strongly expressed GFP in the hippocampus (Wang et al., 2013a). According to previous studies, transgenic upregulation of αCaMKII in the forebrain could cause aggression and anxiety-like behavior in mice, indicating a correlation between αCaMKII level and the state of emotion (Hasegawa et al., 2009). Overexpression of αCaMKII in the medial prefrontal cortex and DG impaired behavioral flexibility and NMDAR-dependent long-term depression, respectively (Ma et al., 2015; Yin et al., 2017b). Other studies also reported that overexpression of the active form of CaMKII in CA1 of the hippocampus might impair context discrimination—a feature also related to PTSD (Ye et al., 2019). Single immobilization stress was found to increase phospho-CaMKII levels in the hippocampus, though there was no change in hippocampal levels of CaMKII (Suenaga et al., 2004). However, following single prolonged stress exposure, considered an animal model of PTSD, the histone deacetylase inhibitor, vorinostat, ameliorated impaired fear extinction. This effect was partly mediated by increased expression of hippocampal CaMKII (Matsumoto et al., 2013).

In the current study, we aimed to focus on the role of dDG αCaMKII in PTSD because the dDG was implicated in defining stress vulnerability or resilience (Albrecht et al., 2017b, 2021b; Ardi et al., 2016; Fa et al., 2014; Ritov et al., 2016; Tripathi et al., 2021).

The lifetime prevalence of exposure to severely stressful events like combat, accidents, natural disasters, assault, or rape is as high as 75–80%, yet only about 10–20% of the population exposed to these stressors will eventually develop PTSD (Kessler et al., 2005b). As in humans, not all animals develop PTSD-like symptoms following trauma exposure. Searching for neural mechanisms associated with the pathology or resilience requires separating the affected individuals who did not develop pathology following trauma exposure (Richter-Levin and Sandi, 2021). Toward that end, the behavior profiling analysis approach was developed. Separating affected and unaffected animals were found pivotal, enabling the identification of individual responses to trauma which would have otherwise been masked by the analysis of averaged group responses (Albrecht et al., 2021a; Ardi et al., 2016; Cohen et al., 2012; Regev-Tsur et al., 2020). We have developed an advanced, algorithm-based behavioral profiling analysis based on previous findings. The analysis of the behavioral outcome of exposure to JS-UWT corroborates previous findings, demonstrating an increased percentage of affected individuals following the combined exposure to JS as a risk factor and to trauma in adulthood (Albrecht et al., 2017a).

Here, we employed two complementary manipulations to examine the role of dDG αCaMKII in trauma vulnerability or resilience: local pharmacological inhibition of CaMKII before UWT exposure and reduction of αCaMKII expression by a viral vector. The reasons behind the inhibition of CaMKII in the DG were that the role of CaMKII in the reconsolidation of memory was unclear in previous studies and never tested for traumatic memories. (Arguello et al., 2014; Jarome et al., 2016; Sakurai et al., 2007). Reducing the expression of dDG αCaMKII could not block the induction of LTP in the DG. However, in another set of animals, reducing the expression of αCaMKII could block the induction of LTP when applying a strong TBS protocol (Fig. 4E). This result agrees with the notion that Strong TBS enables the induction of an αCaMKII-independent form of LTP.

Accumulating evidence indicates that there are different forms of LTP with distinct associated mechanisms (Bauer et al., 2002; Edelmann et al., 2017; III et al., 2004; Morgan and Teyler, 2001; Shin et al., 2010). αCaMKII-dependent LTP may be the dominant form of LTP in the hippocampus, particularly in CA1 (Sweatt, 2016), but other forms of LTP are known (Cook et al., 2006; Wu et al., 2006), which may contribute to plasticity when αCaMKII is blocked or when its expression is inhibited. Plasticity in the DG in the form of LTP has been shown to be alternatively mediated by CaMKII and other pathways, such as PKA/ERK (Cook et al., 2006; Wu et al., 2006). For example, CaMKII inhibitors (KN62 or KN93) did not block LTP in rat DG slices. Nevertheless, when KN62 or KN93 were applied together with the PKA inhibitor (KTS/270) or the MEK inhibitor (Ro-8-cAMP), DG-LTP was blocked entirely, indicating the existence of both αCaMKII-dependent and αCaMKII-independent forms of LTP (Cook et al., 2006; Wu et al., 2006). Having several plasticity-related mechanisms may suggest that different conditions may recruit one or the other mechanisms, and even suggest that there could be a competition between such mechanisms. Such a dynamic range of mechanisms of plasticity fits well with the dynamic role attributed to the DG in defining the outcome of exposure to stress and trauma (Fa et al., 2014). While the CA1 was found to be sensitive to stress, but not to the characteristics of the stressor, the DG was found to be sensitive to both (Bergado et al., 2011; Fa et al., 2014). Our current results provide support to the notion that CaMKII-dependent LTP dominates plasticity in the DG under normal conditions, but that blocking CaMKII might shift the balance towards recruiting other mechanisms that may nevertheless enable plasticity.

We suggest that under normal conditions, the activation of αCaMKII has a dual effect, supporting the formation of αCaMKII-dependent LTP and inhibiting other forms of LTP (Fig. 5A). As a result, TBS leads mainly to the formation of αCaMKII-dependent LTP. Exposure to trauma tends to suppress both αCaMKII-dependent LTP and αCaMKII-independent LTP (Fig. 5B), with an overall result of suppressing LTP. The inhibition of the expression of αCaMKII reduces the probability of activating data suggest that stress can impair cellular plasticity (McEwen and Magarinos, 2001; Sapolsky et al., 2000) and, under some conditions, suppress plasticity in the dDG (Ardi et al., 2014). Diminished plasticity in the DG-PP pathway may reflect altered hippocampal response, as observed in PTSD patients (Levy-Gigi et al., 2015; Milad et al., 2009). Reducing the expression of αCaMKII in JS-UWT exposed animals prevented the impact of the trauma at both the behavioral and electrophysiological levels, indicating that reduced expression of dDG αCaMKII enabled LTP and a form of trauma resilience.

The results demonstrate an elegant correlation between the behavioral and electrophysiological findings (i.e., exposure to trauma, which resulted in PTSD-like symptoms, also suppresses LTP, while blocking αCaMKII, which reduced PTSD-like symptoms also enabled LTP formation). However, since CaMKII is reported to have an essential role in synaptic plasticity, and inhibiting it should block LTP (Colbran and Brown, 2004; Lisman and Raghavachari, 2015; Rossetti et al., 2017; Wayman et al., 2008), it was surprising that αCaMKII knockdown could lead to recovery of DG LTP in trauma exposed animals. One possible explanation could be that using a strong protocol to induce LTP, as we employed here, enabled the induction of an αCaMKII-independent form of LTP. In order to verify that the result is an outcome of the protocol employed, we tested in a separate group of animals the ability of αCaMKII knockdown to impair LTP when employing a regular protocol. Indeed, LTP was significantly impaired when employing a regular TBS protocol, leading to LTP in control animals (Fig. 4C). This result indicates two important points: First, the viral vector used here is effective and functional. Second, as expected, reducing αCaMKII expression impairs the ability to induce LTP in the dDG. However, in another set of animals, reducing the expression of αCaMKII could not block the induction of LTP when applying a strong TBS protocol (Fig. 4E). This result agrees with the notion that Strong TBS enables the induction of an αCaMKII-independent form of LTP.
αCAMKII-dependent LTP, while at the same time, it may also remove the αCAMKII-induced inhibition of other forms of LTP. In turn, this inhibitory removal increases the likelihood of triggering these other forms of LTP, despite the inhibitory effect of the trauma exposure.

In summary, the current findings reveal that reducing the expression level of αCAMKII, specifically in the dDG, leads to increased resilience in the face of a traumatic experience. The pharmacological blockade of CAMKII by KN93 is clearly not specific to αCAMKII, but observing a similar result with the more specific viral KD indicates that it is indeed a reduction in αCAMKII functioning which is at the heart of the effects observed. These results further emphasize the pivotal role dDG has in stress resilience (Regev-Tsur et al., 2020; Tripathi et al., 2021). More generally, the results further support the unique role the dDG has in mediating the interface between emotional and cognitive functions of the hippocampus (Albrecht et al., 2017a; Fa et al., 2014). Accordingly, directed local interventions within the dDG may potentially reduce PTSD symptoms. The current findings also emphasize the contribution of neural plasticity to the normal functioning of the DG and the hippocampus and the possible contribution of abnormal hippocampal plasticity to trauma-related pathological symptoms. It should be emphasized that We found a correlation between the behavioral effect due to trauma and the effect of αCAMKII on DG LTP. Whether DG LTP is causally linked to behavioral outcomes requires further research. Nevertheless, the results suggest a relatively novel angle of examining hippocampal plasticity, i.e., the role of healthy or pathological competition between different forms of plasticity in trauma-related pathology and stress resilience.

Fig. 5. A schematic model illustrating the versatile role of αCAMKII in trauma induced hippocampal plasticity (A) Under normal conditions, the activation of αCAMKII has a dual effect supporting the formation of αCAMKII-dependent LTP while inhibiting other forms of LTP. As a result, TBS leads mainly to the formation of αCAMKII-dependent LTP. (B) Exposure to trauma tends to suppress both αCAMKII-dependent LTP and αCAMKII independent LTP. (C) Reducing the expression of αCAMKII reduces the probability of activating αCAMKII-dependent LTP. At the same time, it may also remove αCAMKII-induced inhibition of other forms of LTP. Accordingly, it increases the likelihood of inducing these other forms of LTP, despite the inhibitory effect of the trauma exposure.

Funding

This research was supported by MOST China-Israel cooperation (No: 2016YFE01030500) grant 3-13563 to GR-L & XH, by research grant from the State of Israel Ministry of Science, Technology, & Space to GR-L, by research grant 3-14356 from the State of Israel Ministry of Science, Technology, & Space to GR-L.

CRediT authorship contribution statement

Somoday Hazra: Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. Joyeeta Dutta Hazra: Investigation, Visualization. Rani Amit Bar-On: Methodology, Validation. Yanhong Duan: Investigation. Shahaf Edut: Investigation. Xiaohua Cao: Investigation. Gal Richter-Levin: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

Somoday Hazra, Joyeeta Dutta Hazra, Rani Amit Bar-On, Yanhong Duan, Shahaf Edut, Xiaohua Cao and Gal Richter-Levin declare no competing conflicts of interest.

Acknowledgments

We thank Dr. Rachel Anunu for her technical support, Dr. Silvia Mandel for editing the manuscript and Anaam Kraiyim for her assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jynstr.2022.100506.

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