eLife

Reviewed Preprint
v1 • July 10, 2026
Not revised

For correspondence:
camilo@nus.edu.sg
phssks@nus.edu.sg

Competing interests: No competing interests declared

Funding: See page 11

Reviewing editor: Upinder S Bhalla, National Centre for Biological Sciences, India

Primate Hippocampus Reveals Distinct Rules for Associative Synaptic Plasticity

Anoop Manakkadan[1], Krishna Kumar[1], Yee Song Chong[1], Wong Lik-Wei[1], Sheeja Navakkode[2], Yuk Peng Wong[1], Soong Tuck Wah[1,3], Camilo Libedinsky[3,4,5] ✉, Sreedharan Sajikumar[1,2,3] ✉

[1]Department of Physiology, Yong Loo Lin School of Medicine National University of Singapore, Singapore, Singapore • [2]Healthy Longevity Translational Research Programme, Yong Loo Lin School of Medicine National University of Singapore, Singapore, Singapore • [3]Neurobiology Programme, Life Sciences Institute, National University of Singapore, Singapore, Singapore • [4]Department of Psychology, National University of Singapore, Singapore, Singapore • [5]N.1 Institute for Health, National University of Singapore, Singapore, Singapore

eLife Assessment

This is a potentially **important** study comparing LTP mechanisms between primates and rodents. The experimental methods have some possible confounds, and the power (replicates) and design of the statistical methods could be strengthened, hence the support for the central claims of species differences is currently **incomplete**.

https://doi.org/10.7554/eLife.111886.1.sa4

Abstract

Long-term potentiation (LTP) is a key cellular mechanism underlying learning and memory, but its conservation across species remains unclear. Using nonhuman primates (NHPs), we examined hippocampal synaptic plasticity at Schaffer collateral– CA1 synapses. Theta-burst stimulation (TBS) reliably induced LTP in NHPs, comparable to rodents. However, unlike rodents, TBS in NHPs readily engaged synaptic tagging and capture (STC), indicating a lower threshold for associative plasticity. This was accompanied by increased expression of plasticity-related proteins, including PKMζ and BDNF, suggesting enhanced recruitment of protein synthesisdependent stabilization mechanisms. These findings reveal a species-specific divergence in the molecular regulation of persistent synaptic plasticity and identify an evolutionary specialization in mechanisms supporting associative memory. Together, our results highlight limitations of rodent models in fully capturing human-relevant memory processes and underscore the importance of primate systems for translational neuroscience.

Introduction

Memory is widely regarded as an evolutionarily conserved cognitive function, although its complexity varies across species. At the cellular level, memory formation is thought to rely on activity-dependent changes in synaptic strength, commonly referred to as synaptic plasticity. However, the precise mechanisms by which neural circuits stabilize and adapt to these plastic changes to support complex learning remain poorly understood. Recent comparative studies between rodents and non-human primates (NHPs) provide compelling evidence for an evolutionarily conserved memory coding scheme, suggesting that fundamental aspects of information storage may be preserved across species (1). However, differences have also been uncovered between rodents and primates. For example, while the rodent hippocampus contains place cells that encode the animal's location in space, the primate hippocampus contains view cells that encode the animal's gaze direction(2).

Activity-dependent changes in synaptic strength, such as long-term potentiation (LTP), are widely proposed to underlie memory storage in the brains of mammals, including humans (3, 4). Although most studies of synaptic plasticity have been conducted in rodents, evidence from primate brains suggests that the fundamental mechanisms of LTP are conserved across species. Consistent with this, LTP can be reliably induced at both mossy fiber-CA3 and associational-commissural synapses in hippocampal slices from cynomolgus monkeys, with induction properties closely resembling those observed in rodent hippocampus (5). Similarly, NMDAR-dependent LTP has been reported in human cortical tissue (6) and in the primate visual cortex, where it exhibits Hebbian and associative properties comparable to those described in rodents (7). Moreover, in vivo studies in awake monkeys have demonstrated long-lasting spike-timing-dependent plasticity in the sensorimotor cortex, further supporting the presence of conserved synaptic plasticity mechanisms in primate brains (8). However, despite these similarities, it remains unclear whether differences exist in the mechanisms governing associative synaptic plasticity across species, an important question that warrants further investigation.

One of the most compelling aspects of LTP lies in the molecular mechanisms that support its persistence, a feature considered fundamental to memory storage. Importantly, LTP is not a unitary phenomenon; its expression varies depending on the specific synapse and neural circuits involved. Distinct patterns of stimulation can give rise to mechanistically different forms of LTP (9). Among these, theta burst stimulation (TBS) and high-frequency stimulation (HFS) are two commonly employed protocols for inducing LTP (10). Although both paradigms engage overlapping molecular targets, TBS-induced LTP (TBS-LTP) is particularly sensitive to various experimental manipulations, including aging (11), stress (12), serotonin modulation (13), endocannabinoid signaling (14), and adenosine receptor activity (15). This heightened sensitivity makes TBS-LTP a potentially more informative model for examining and comparing LTP mechanisms across species. Accordingly, TBS-LTP offers a physiologically relevant and experimentally advantageous platform for comparative analyses of synaptic plasticity across species.

TBS-LTP is particularly well suited for translational comparisons, as it is largely dependent on local protein synthesis, thereby minimizing nuclear involvement and emphasizing synapse-autonomous mechanisms. Moreover, LTP induced by TBS fails to engage associative plasticity mechanisms such as synaptic tagging and capture (STC) in rats (16, 17). STC refers to a process in which weakly activated synapses set transient molecular "tags" that allow them to capture plasticity-related proteins (PRPs) synthesized in response to a strong, temporally proximal stimulus, thereby stabilizing long-term synaptic changes (3, 18, 19). The inability of TBS-LTP to recruit STC may reflect insufficient availability of key PRPs at tagged synapses, such as PKMζ or a failure of TBS to elicit cell-wide protein synthesis and distribution necessary for associative synaptic consolidation (16).

Metaplasticity, the plasticity of synaptic plasticity, has been shown to modulate the mechanisms underlying STC through the recruitment of distinct PRPs (16, 20). By dynamically adjusting the threshold for LTP, metaplasticity establishes an optimal synaptic state for associative learning. Importantly, the LTP modification threshold is likely to vary across species, reflecting differences in the intrinsic complexity of neural circuits and regulatory processes. In support of this notion, comparative synaptic proteomic analyses of the hippocampus have revealed pronounced differences in protein expression profiles between rodents and primates (21). These observations raise the possibility that species-specific expression of distinct synaptic proteins may underlie functional differences in cellular associative properties across mammals.

In this study, we show that TBS reliably induces robust and persistent LTP lasting up to 6 hours in hippocampal slices from both rodents and NHPs. Strikingly, whereas TBS-induced LTP in rats failed to engage associative plasticity, the same stimulation paradigm facilitated STC in primates. Consistent with this divergence, TBS elicited higher expression levels of *BDNF* and *CaMKIV* transcripts in primate hippocampus compared with rats. At the protein level, Western blot analyses revealed a pronounced upregulation of PKCζ and BDNF following TBS-LTP in primates, an effect that was absent in rodent hippocampal slices and control NHP tissue. Pharmacological

studies further uncovered species-specific mechanisms of LTP maintenance. In rats, BDNF was required for the persistence of LTP but did not contribute to its associative expression. In contrast, in primates, BDNF and PKMζ exhibited functional redundancy, such that inhibition of either molecule alone failed to disrupt LTP maintenance. However, simultaneous inhibition of both BDNF signaling and PKMζ activity abolished the late phase of LTP, revealing an interdependent molecular framework that sustains synaptic potentiation in the primate hippocampus.

Results

TBS-Induced LTP Promotes Synaptic Tagging and Capture in NHP Hippocampal Slices

To examine species specificity in the induction of TBS-LTP, we used hippocampal slices from rats and monkeys. Using a two-input experimental model (Fig. 1A i-iii), we show that a 5-Hz theta-burst stimulation (TBS) protocol reliably induced late-phase LTP (L-LTP) in both rat and NHP hippocampal slices. In rodents, this form of LTP has been shown to depend on protein translation but not transcription (17).

As a control experiment, we first investigated TBS-LTP in rat hippocampal slices. After recording a stable baseline for 30 min, TBS-LTP was induced in synaptic input S1 (Fig. 1B, filled blue circles). Statistically significant potentiation was observed at Schaffer collateral-CA1 synapses in rats starting from the first recording after TBS, at 5 min (Wilcox test, $P = 0.0313$; U-test, $P = 0.0022$), and persisted until 6 h (Wilcox test, $P = 0.0313$; U-test, $P = 0.0022$), compared to its own baseline or the control input S2. Baseline responses recorded from synaptic input S2 remained stable (Fig. 1B, open blue circles).

Given the involvement of the primate hippocampus in higher-order cognition, we reasoned that neural activity patterns may differ between NHPs and rodents. Accordingly, we tested whether the NHP hippocampus exhibits similar thresholds for TBS-induced LTP. As in Fig. 1C, a stable baseline was recorded for 30 min in both S1 and S2 (filled and open brown circles), after which TBS was delivered to input S1. TBS induced a potentiation that was statistically significant from 5 min up to 6 h (Wilcox test, $P = 0.0313$; U-test, $P = 0.0022$). These results demonstrate that, similar to rats and mice, TBS can induce a persistent potentiation lasting up to 6 h in NHP hippocampal slices.

As activity-induced synaptic plasticity was comparable in rat and NHP hippocampal slices, we next asked whether short-term potentiation, such as early-LTP, is similarly preserved in NHPs. In rat hippocampal slices, early-LTP was induced using a WTET protocol (Fig. 1D). WTET elicited a transient potentiation that persisted only until 155 min relative to its own baseline (Wilcox test, $P = 0.0313$) and until 155 min when compared with the non-tetanized S2 pathway (U-test, $P = 0.0095$, Fig.1D, filled blue circles). To examine short-term synaptic plasticity in NHP hippocampal slices, WTET was next delivered to S1 (Fig. 1E). In this case, WTET induced an LTP that was significant only until 155 min relative to its own baseline (Wilcox test, $P = 0.0313$) and 155 min when compared with S2 (U-test, $P = 0.0022$) Fig.1E, filled brown circles).

Previous studies have reported that TBS-induced LTP is unable to support the expression of STC (16, 17). To verify this, STC was examined in rat hippocampal slices (Fig. 1F). TBS-LTP was induced in input S1 (filled blue circles), and 30 min later WTET was delivered to S2 (open blue circles) to induce early-LTP. Consistent with earlier findings, no expression of STC was observed. Potentiation in S1 remained statistically significant for up to 6 h (Wilcox test, $P = 0.0156$), whereas in S2 late-LTP was not expressed and synaptic responses decayed to baseline within 200 min (Wilcox test, $P = 0.0625$).

Given that rodent hippocampal slices fail to express STC during TBS-induced activity, we next asked whether TBS-LTP in NHP hippocampal slices can engage STC. After a stable 30-min baseline recording, TBS was applied to input S1 (Fig. 1G, filled brown circles). Thirty minutes later, early-LTP was induced in S2 by WTET (Fig. 1G, open brown circles). Strikingly, early-LTP in S2 was transformed into late-LTP, indicating robust expression of STC. Both S1 and S2 exhibited significant

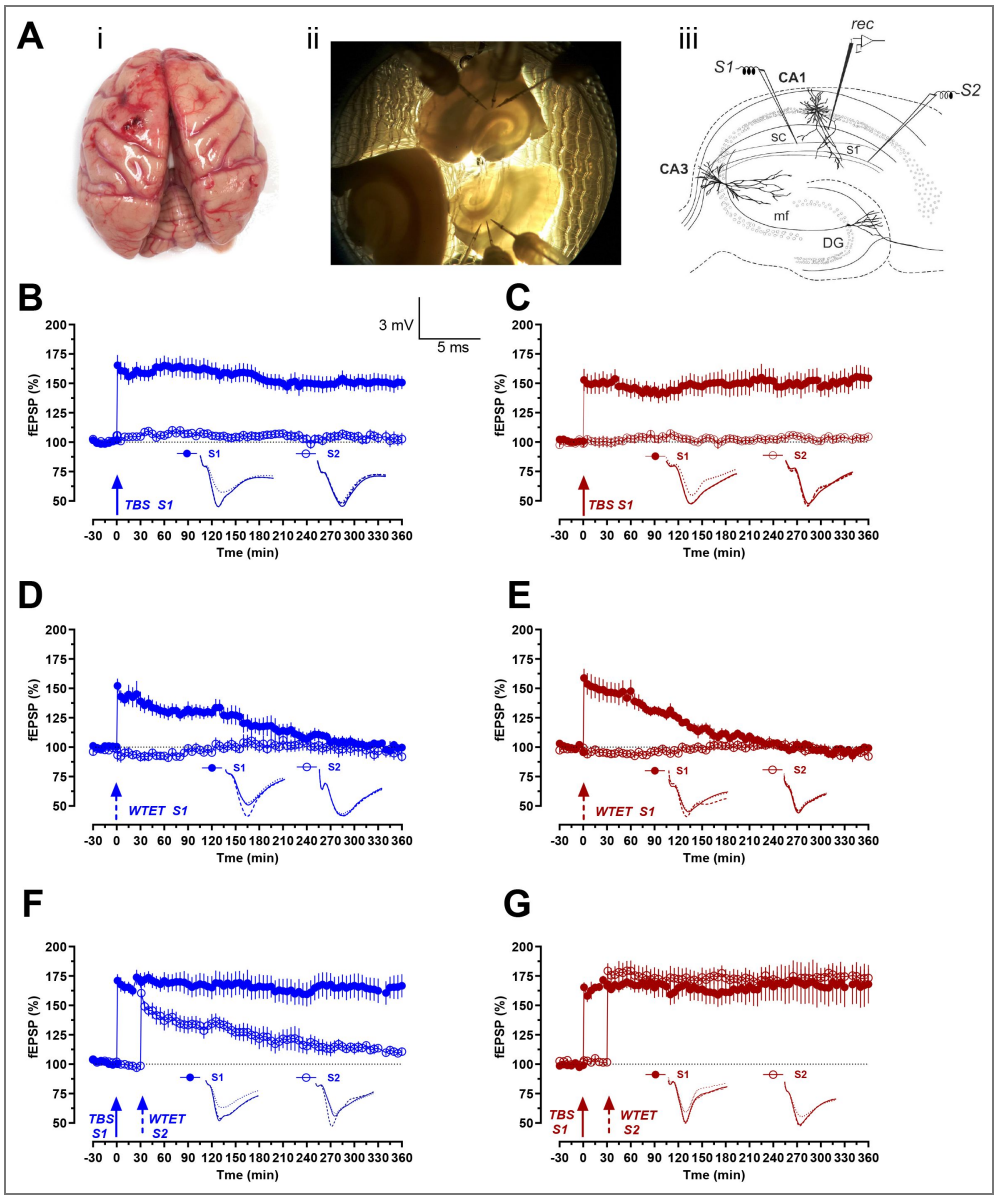

Figure 1. Species-specific differences in TBS-induced synaptic plasticity and synaptic tagging and capture (STC) in hippocampal CA1.

(A) (i) Isolated NHP brain. (ii) Representative image of NHP hippocampal slice preparation with electrodes positioned in the CA1 region. (iii) Schematic of a hippocampal slice illustrating two independent synaptic inputs (S1 and S2) onto CA1 pyramidal neurons. Field excitatory postsynaptic potentials (fEPSPs) were recorded from apical dendrites in CA1 using a recording electrode (rec). **(B-C)** TBS-induced late-phase LTP (late-LTP). Following a stable 30 min baseline, theta-burst stimulation (TBS; 5 Hz) delivered to input S1 induced robust and persistent potentiation in both rat (B) and NHP (C) hippocampal slices. Potentiation was significant from 5 min post-TBS and persisted up to 6 h, whereas the control pathway S2 remained stable. **(D-E)** Early-LTP induced by weak tetanic stimulation (WTET). WTET applied to S1 induced transient potentiation in both rat (D) and NHP (E) hippocampal slices. Early-LTP decayed to baseline within ~155-200 min, with no sustained potentiation observed in either species. **(F-G)** In rat hippocampal slices (F), TBS-induced late-LTP in S1 failed to support STC, as WTET-induced potentiation in S2 decayed to baseline, indicating lack of plasticity-related protein (PRP) capture. In contrast, in NHP hippocampal slices (G), WTET delivered to S2 30 min after TBS in S1 resulted in the conversion of early-LTP into persistent late-LTP, demonstrating robust STC expression. Both S1 and S2 pathways remained significantly potentiated throughout the 6 h recording period. In all panels, figures shown in blue represent experiments conducted in rats, whereas those in dark red represent experiments in nonhuman primates (NHPs). Solid and dotted arrows indicate the time points of application of TBS and WTET, respectively. Analog traces depict representative S1 and S2 fEPSPs: 30 min before any experimental manipulation (dotted line), 30 min (hatched line) and 360 min (solid line) after TBS or WTET. Vertical scale bar: vertical: 3 mV, horizontal scale bar: 5 ms.

potentiation immediately following TBS and remained potentiated throughout the entire 6-h recording period (Wilcox test, $P = 0.0313$ for S1; Wilcox test, $P = 0.0313$ for S2 at 360 min post-tetanization).

Together, these findings demonstrate that stable and long-lasting LTP can be induced in NHP hippocampal slices and, importantly, that TBS-LTP in NHPs supports STC. This suggests that NHP hippocampal circuits may possess a distinct proteomic environment that facilitates the synaptic tagging and capture of plasticity-related proteins.

Divergent Gene Expression and Proteomic Signatures in Rat and Non-Human Primate Hippocampi

It is well established that BDNF, CaMKII, CaMKIV, PKMζ, and CREB play critical roles in the maintenance of long-term synaptic plasticity in the hippocampal CA1 region of rodents (3). We therefore assessed mRNA expression levels in rat and NHP hippocampal slices under control conditions and following TBS-induced LTP (Fig.2). In NHP hippocampal slices, TBS-LTP resulted in a significant increase in BDNF and CaMKIV mRNA expression in the CA1 region compared with control slices without TBS. In contrast, the expression levels of CaMKII, PKMζ, and CREB did not show significant changes (Fig.2A). Interestingly, TBS did not induce significant changes in the expression of any of the candidate molecules in rat hippocampal slices (Fig. 2B). In NHP hippocampal slices, TBS-LTP induced a significant increase in BDNF (t test, $P = 0.0234$) CaMKIV (t test, $P = 0.0362$) mRNA expression in the CA1 region compared with control slices without TBS (Fig. 2A). In contrast, the expression levels of CaMKII ($P = 0.0786$), PKMζ ($P = 0.3657$), and CREB1 ($P = 0.5700$) were not significantly changed. In rat hippocampal slices, TBS did not produce significant changes in the expression of any of the examined molecules (Fig. 2B), including BDNF ($P = 0.2495$), CaMKII ($P = 0.9195$), CaMKIV ($P = 0.5554$), PKMζ ($P = 0.9407$), and CREB1 ($P = 0.2794$).

Given the established roles of PKMζ and BDNF in maintaining long-term plasticity and synaptic tagging and capture in hippocampal CA1 (16), we next examined their protein expression levels in rat and NHP hippocampi following TBS. TBS induced a significant increase in both PKMζ (t test, $P = 0.0221$) and BDNF (t test, $P = 0.0184$) protein levels in NHP hippocampus but not in rat hippocampus (Fig. 2C-D-E). These results suggest that TBS-induced up-regulation of PKMζ in the NHP hippocampus may contribute to the enhanced STC observed in NHPs compared with rodents.

The Roles of BDNF and PKMζ in LTP maintenance in rats and NHPs

It has been previously reported that blocking PKMζ 1 h after tetanization reverses established LTP (22, 23), whereas inhibition of PKMζ after tetanization fails to disrupt potentiation in DHPG-primed TBS-LTP (16). We first examined whether BDNF contributes to TBS-LTP in rat hippocampal slices. To this end, recombinant human TrkB/Fc chimera (TrkB/Fc; 1 μg/mL) was applied 60 min after the induction of TBS-LTP and maintained until the end of the experiment (Fig.3A). TrkB/Fc scavenges extracellular BDNF and thereby blocks TrkB activation. Blockade of BDNF impaired the late phase of LTP (Fig. 3A, filled blue circles), resulting in a decaying potentiation that persisted only up to 250 min relative to its own baseline (Wilcoxon test, $P = 0.0313$) and until 250 min when compared with S2 (U-test, $P = 0.0238$).

We next assessed the role of BDNF in NHP TBS-LTP by applying TrkB/Fc (1 μg/mL) 60 min after TBS-LTP induction and continuing the application until the end of the recording (Fig. 3C). Surprisingly, BDNF blockade had no significant effect on LTP maintenance in NHP hippocampal slices (Fig. 3C, filled brown circles), resembling the BDNF independence previously reported for DHPG-primed TBS-LTP (16). Potentiation in S1 was statistically significant immediately after TBS and remained stable throughout the entire 6-h recording period (Wilcoxon test, $P = 0.0313$; U-test, $P = 0.0022$ at 360 min post-tetanization). We then investigated the role of PKMζ in TBS-induced LTP in both rat and NHP hippocampal slices. Application of a PKMζ inhibitor (PKMZi) 60 min before TBS-LTP induction and maintained throughout the remaining recording period had no effect on TBS-LTP maintenance in either species (Fig. 3B and 3D). In both cases, LTP persistence after

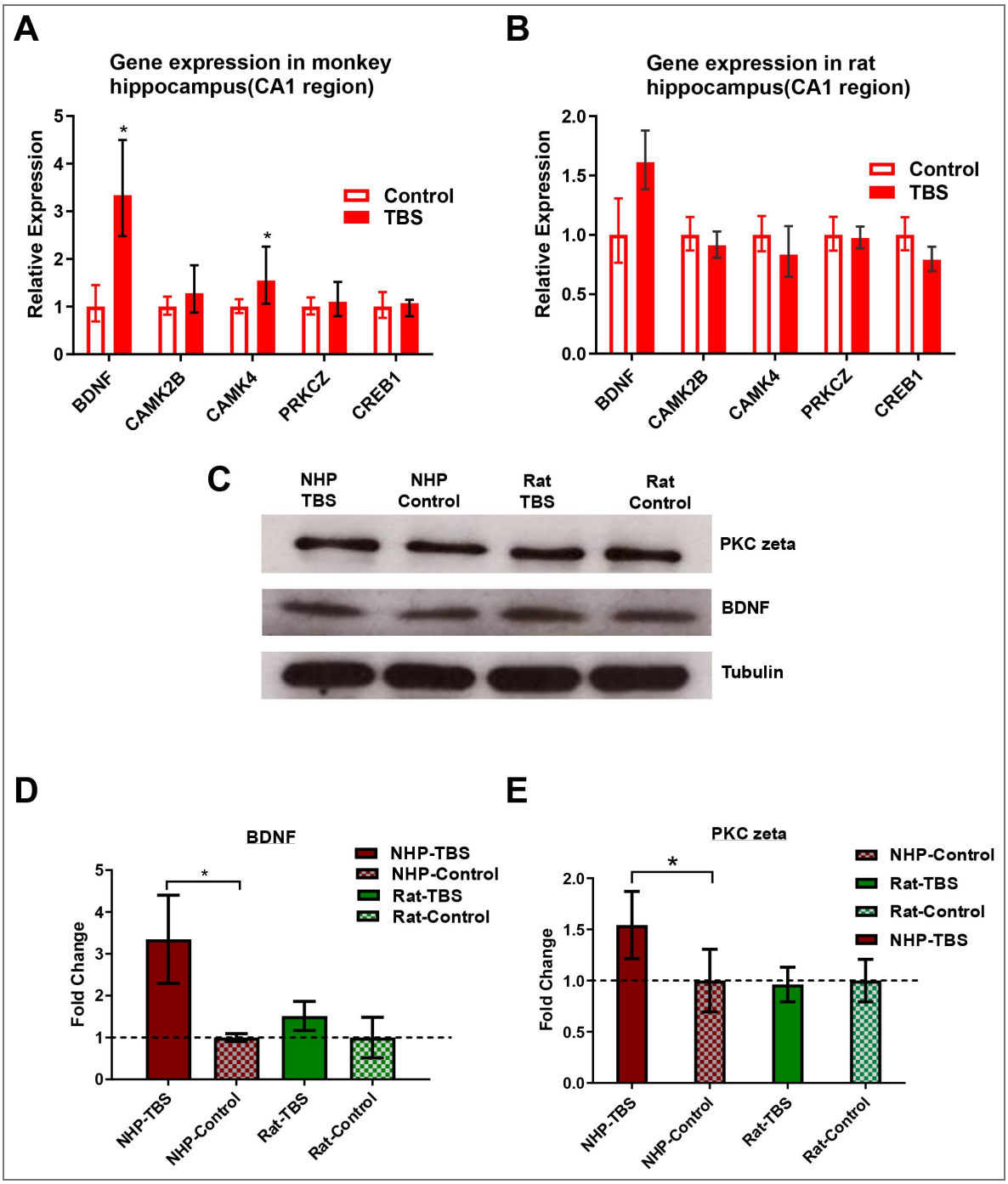

Figure 2. Divergent gene expression and proteomic signatures in rat and nonhuman primate (NHP) hippocampal CA1 following TBS-induced LTP.

(A-B) Relative mRNA expression levels of plasticity-related genes in hippocampal CA1 under control conditions (black) and following TBS-induced LTP (red). (A) In NHP hippocampal slices, TBS significantly increased the expression of *BDNF* and *CaMKIV*, while *CaMKII, PKMζ*, and *CREB1* remained unchanged. (B) In rat hippocampal slices, TBS did not induce significant changes in the expression of any of the examined genes. **(C)** Representative Western blot images showing protein levels of PKMζ and BDNF in NHP and rat hippocampal slices under control conditions and following TBS. Tubulin was used as a loading control. **(D-E)** Quantification of protein expression levels. (D) BDNF and (E) PKMζ protein levels were significantly increased following TBS in NHP hippocampal slices compared to controls, whereas no significant changes were observed in rat hippocampal slices.

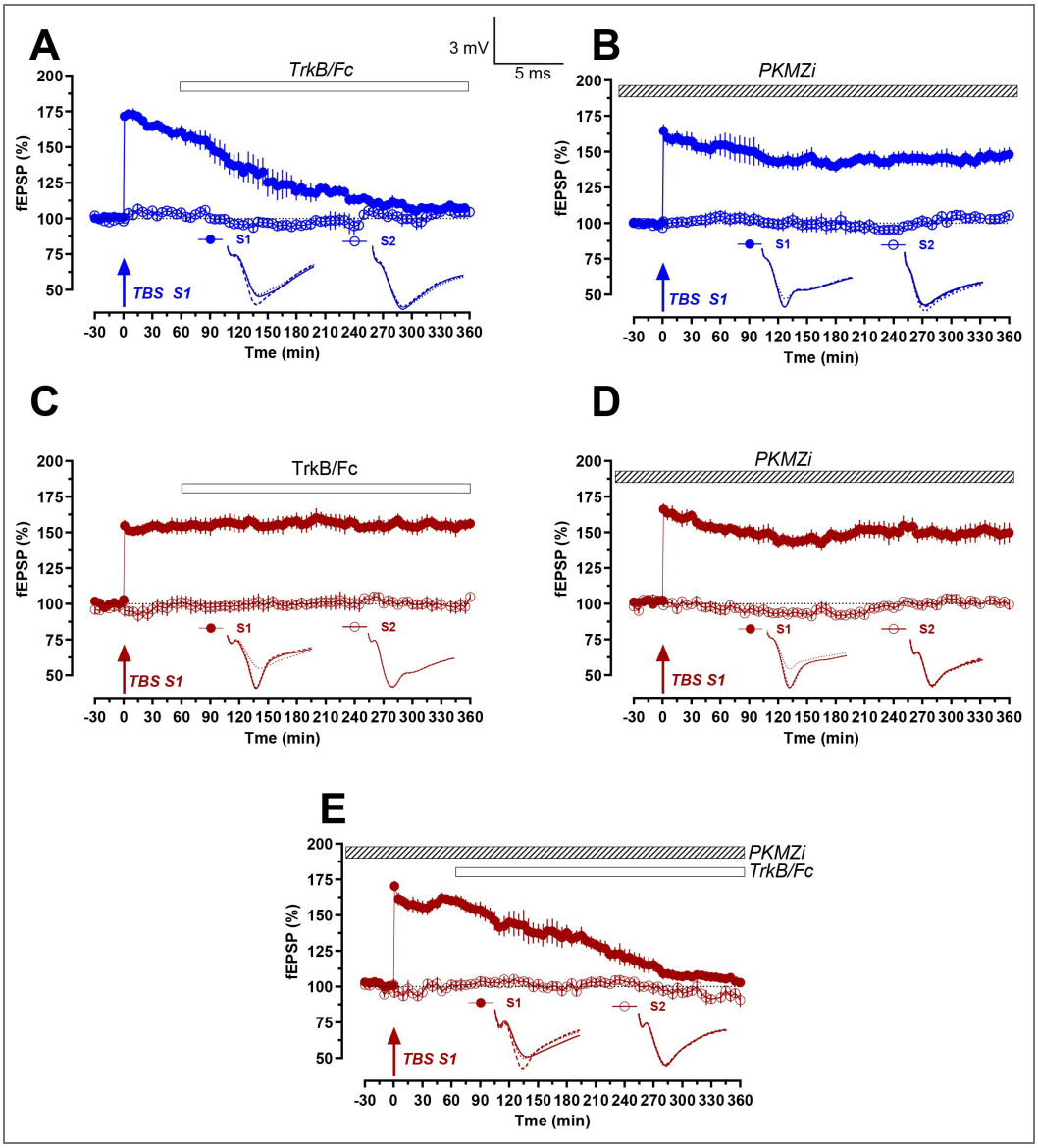

Figure 3. Differential roles of BDNF and PKMζ in the maintenance of TBS-induced LTP in rat and nonhuman primate (NHP) hippocampal CA1

(A-B) Rat hippocampal slices. (A) Blockade of BDNF signaling using TrkB/Fc applied 60 min after TBS impaired the late phase of LTP, resulting in a gradual decay of potentiation in S1, while the control pathway S2 remained stable. (B) In contrast, inhibition of PKMζ (PKMZi), applied prior to TBS and maintained throughout the recording, did not affect LTP maintenance, indicating that PKMζ is not required for TBS-induced LTP in rat CA1 and that it can be maintained exclusively by BDNF. **(C-D)** NHP hippocampal slices. (C) Inhibition of BDNF signaling with TrkB/Fc did not disrupt TBS-induced LTP, with S1 responses remaining persistently potentiated throughout the recording period. (D) Similarly, inhibition of PKMζ alone did not affect LTP maintenance, indicating that TBS-induced LTP in NHP hippocampus is resistant to disruption of either pathway individually. Asterisks denote statistically significant differences between groups (*P < 0.05). **(E)** Combined inhibition of BDNF and PKMζ in NHP hippocampal slices. Simultaneous blockade of both pathways prevented the maintenance of TBS-induced LTP, resulting in a decay of potentiation in S1 toward baseline levels, while S2 remained stable. This indicates cooperative and partially redundant roles of BDNF and PKMζ in sustaining long-term synaptic plasticity in NHP CA1. In all panels, figures shown in blue represent experiments conducted in rats, whereas those in dark red represent experiments in nonhuman primates (NHPs). Solid arrow indicate the time points of application of TBS. A hatched or empty rectangle represents the timing and duration of application of specific inhibitors. Analog traces depict representative S1 and S2 fEPSPs: 30 min before any experimental manipulation (dotted line), 30 min (hatched line) and 360 min (solid line) after TBS or WTET. Vertical scale bar: vertical: 3 mV, horizontal scale bar: 5 ms.

TBS remained significantly elevated relative to the corresponding baseline and the independent control input S2 (Wilcoxon test, $P = 0.0313$; U-test, $P = 0.0022$ for rat; Wilcoxon test, $P = 0.0313$; U-test, $P = 0.0022$ for NHP at 360 min post-tetanization).

Thus, whereas inhibition of BDNF selectively disrupts the late phase of TBS-LTP in rat hippocampal slices while sparing PKMζ-dependent mechanisms, TBS-LTP in NHP slices is resistant to inhibition of either BDNF or PKMζ alone. We therefore tested the combined effect of BDNF and PKMζ inhibition (Fig. 3E). Strikingly, simultaneous inhibition of BDNF and PKMζ prevented the maintenance of TBS-LTP in NHP hippocampal slices (filled brown circles), with statistically significant potentiation lasting only up to 245 min relative to its own baseline (Wilcoxon test, $P = 0.0313$) and up to 270 min when compared with the independent control input S2 (U-test, $P = 0.0411$).

Together, these findings indicate that TBS-LTP in NHP hippocampus is supported by cooperative and partially redundant molecular mechanisms that differ fundamentally from those operating in rodent hippocampus.

Discussion

Our findings demonstrate that TBS induces persistent LTP in both rodent and NHP hippocampal slices. TBS closely mimics endogenous theta-frequency firing patterns associated with exploratory behavior and memory encoding and is therefore widely used as a physiologically relevant model of synaptic plasticity (24, 25). In rodents, TBS-induced LTP is largely localized, translation-dependent, and relatively independent of transcription, resulting in spatially restricted availability of plasticity-related proteins (PRPs) (26-29). This compartmentalization limits its ability to support synaptic tagging and capture (STC), which requires broader PRP availability (16).

In contrast, we observed that TBS-LTP in NHP hippocampus facilitates STC, indicating a fundamental divergence in the mechanisms underlying LTP persistence across species. Importantly, the threshold for inducing short-lasting LTP via weak tetanization was comparable between rodents and NHPs, suggesting that baseline synaptic excitability is conserved. Therefore, the observed differences are unlikely to arise from altered induction thresholds but instead reflect divergence in downstream molecular processes governing PRP availability and synaptic stabilization.

A key finding of this study is the differential recruitment of PRPs following TBS. In rodents, TBS-LTP critically depends on BDNF signaling, as BDNF sequestration abolishes the expression of TBS-LTP (16, 27). Consistent with this, inhibition of PKMζ does not affect LTP maintenance in rats, suggesting that PKMζ is not recruited following TBS-induced potentiation (16, 23). In contrast, our data show that TBS in NHP hippocampus leads to increased expression of both BDNF and PKMζ. Given that PKMζ is a key determinant of PRP availability and synaptic stabilization, its recruitment in NHPs may lower the threshold for STC and facilitate persistent plasticity. This is consistent with previous observations in primed LTP paradigms, such as DHPG-facilitated TBS-LTP, where enhanced PRP synthesis supports long-lasting potentiation (16). However, simultaneous inhibition of both pathways abolished LTP maintenance, indicating that BDNF and PKMζ may function in a compensatory or cooperative manner in primates. This redundancy may enhance the robustness and flexibility of synaptic plasticity in species with more complex cognitive capabilities.

Molecular analyses further revealed species-specific differences in gene and protein expression profiles following TBS. In NHPs, elevated mRNA levels of BDNF and CaMKIV indicate a greater contribution of transcription-dependent mechanisms to LTP maintenance, whereas such changes are not observed in rodents under TBS. At the protein level, elevated PKCζ and BDNF in NHPs further support enhanced PRP availability. These findings are consistent with previous studies demonstrating divergence in the expression and regulation of PRPs between rodents and primates, despite conservation of core synaptic machinery (21). Beyond molecular mechanisms, structural and circuit-level differences between rodent and primate brains-including divergence in synaptic organization, connectivity, and neuronal diversity-may also contribute to the observed effects (21, 30). Variations in neuronal density, synaptic organization, and interneuron diversity

can influence network dynamics and integration properties (31, 32). Additionally, primate-specific transcriptional programs and epigenetic regulatory mechanisms may modulate activity-dependent gene expression, including plasticity-related genes such as Arc, Egr1, and c-Fos (33, 34). These differences may extend the temporal window for PRP availability and enhance the efficiency of synaptic tagging and capture; however, this remains to be investigated, particularly in light of recent rodent evidence suggesting that tag–PRP interactions can persist longer than originally proposed (35).

Collectively, our findings support the view that STC represents a conserved framework for memory-related plasticity, but that the molecular pathways governing PRP production and utilization have undergone species-specific specialization. In rodents, TBS-LTP relies predominantly on BDNF-dependent mechanisms, whereas in primates, additional recruitment of PKMζ and transcriptional pathways enhances the probability of long-term synaptic stabilization.

In conclusion, our study demonstrates that physiologically patterned stimulation engages distinct molecular programs in the rodent and NHP hippocampus, leading to differences in the associativity of synaptic plasticity. These findings reveal fundamental differences in the molecular architecture underlying plasticity across species and underscore the importance of incorporating NHP models in translational neuroscience research. Reliance solely on rodent systems may overlook critical regulatory mechanisms relevant to human cognition and memory disorders. Therefore, understanding species-specific molecular cascades, particularly those governing STC, will be essential for translating synaptic plasticity mechanisms to human cognition and for developing effective therapeutic strategies targeting memory disorders.

Materials and Methods

Preparation of hippocampal slices

A total of 36 acute hippocampal slices were obtained from 7 male Macaca fascicularis (5-7years old), and 31 hippocampal slices were obtained from 15 male Wistar rats (5-7 weeks old). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore.

Briefly, rodents were decapitated after anesthetization using CO_2. NHPs were euthanized using pentobarbital (150 mg/kg). Adequate euthanasia was confirmed by cyanosis of the mucous membranes and the absence of palpebral reflex, heart rate, and respiratory movements., In both cases, the brains were quickly removed and cooled in 4°C artificial cerebrospinal fluid (ACSF) that contained the following (in mM): 124 NaCl, 3.7 KCl, 1.0 $MgSO_4$.$7H_2O$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 24.6 $NaHCO_3$ and 10 D-glucose, equilibrated with 95% O_2–5% CO_2 (carbogen; total consumption 16 L/hr), and acute hippocampal slices were prepared from the right hippocampus using a manual tissue chopper. Hippocampal slices were then transferred onto the interface brain slice chamber (Scientific Systems Design) and incubated for three hours at 32°C with ACSF before the electrophysiology studies In all electrophysiological recordings, two-pathway experiments were conducted. Two monopolar lacquer-coated stainless steel electrodes (5 MΩ; AM Systems, Sequim) were positioned at an appropriate distance within the stratum radiatum of the CA1 region to stimulate two independent synaptic inputs, S1 and S2, within a single neuronal population. These electrodes elicited field excitatory postsynaptic potentials (fEPSPs) from Schaffer collateral/commissural-CA1 synapses (Figure 1a). Pathway specificity was confirmed following the method described by (16). A third electrode (5 MΩ; AM Systems) was placed in the apical dendritic layer of the CA1 region to record fEPSP. The signals were amplified using a differential amplifier (Model 1700; AM Systems), digitized with a CED 1401 analog-to-digital converter (Cambridge Electronic Design), and monitored online. After the pre-incubation period, a synaptic input-output curve (afferent stimulation vs. fEPSP slope) was generated. The test stimulation intensity was adjusted to evoke an fEPSP slope corresponding to 40% of the maximal response for both S1 and S2 synaptic inputs. To maximize the efficient use of NHP hippocampal tissue, electrophysiological recordings were performed using three interface chambers, each accommodating two slices for simultaneous recordings. Distinct stimulation paradigms were

applied concurrently across chambers, enabling multiple experimental conditions to be examined in parallel within the same biological preparation. This approach allowed systematic comparison of experimental manipulations while minimizing the number of animals required.

Late long-term potentiation (L-LTP) was induced using a theta-burst stimulation (TBS) protocol similar to that described previously (16). The protocol consisted of 50 bursts, each containing four stimuli delivered at an interstimulus interval of 10 ms. The bursts were applied at 5 Hz (inter-burst interval of 200 ms) over a period of 10 s (16). For early LTP induction, a "weak" tetanization (WTET) protocol comprising a single stimulus train of 21 pulses at 100 Hz (0.2 ms stimulus duration per polarity) was used, as described by (36). In all experiments, a stable baseline was recorded for at least 30 minutes using four biphasic constant-current pulses (0.1 ms per polarity) delivered at 0.2 Hz at each time point.

Pharmacology

The PKMζ antisense oligodeoxynucleotide (IDT, Singapore) was stored as a 2 mM stock solution in Tris-EDTA (TE, pH 8.0) buffer at −20 °C (Tsokas et al., 2016). Stock solutions were stored for no longer than one week. Before use, the stock was diluted to the required final concentration in artificial cerebrospinal fluid (ACSF), bubbled with carbogen (95% O_2 / 5% CO_2), and bath-applied for the specified duration. The final concentration of PKMζ antisense oligodeoxynucleotide used in the experiments was 20 μM.

TrkB-Fc chimeric protein (Cat. #688-TK, R&D Systems, Minneapolis, MN, USA) was prepared fresh for each experiment by dissolving the lyophilized protein in distilled water (dH_2O) to obtain a final concentration of 1 μg/mL.

mRNA Quantitative Real-Time PCR

For mRNA expression analysis, cDNA synthesis was performed using the GoScript Reverse Transcription System (Promega, USA). Briefly, 2 μg of RNA was preheated with 2 μL of Oligo (dT) at 72°C for 2 minutes. Reverse transcription was conducted at 42°C for 1 hour, followed by enzyme inactivation at 95°C for 5 minutes. Quantitative real-time PCR (qRT-PCR) was carried out using the StepOne Plus Real-Time PCR System (Applied Biosystems) with TaqMan Universal PCR Master Mix (Cat. No. 4304437; Thermo Scientific) and TaqMan probes specific for BDNF, CaMKII, CaMKIV, PKMζ and CREB. The qRT-PCR protocol involved an initial denaturation step at 95°C for 10 minutes, followed by 40 amplification cycles of 95°C for 15 seconds and 60°C for 1 min. The fold changes in BDNF, CaMKII, CaMKIV, PKMζ and CREB. gene expression were calculated using the $2^{-}\Delta\Delta Ct$ method (Livak & Schmittgen, 2001), with GAPDH serving as the internal normalization control. Each sample was measured in duplicate, and gene expression analysis was performed on hippocampal slices obtained from three independent biological variants in each group (rodent or NHP; n = 3).

Western blot analysis

In brief, hippocampal slices from four experimental groups (NHP TBS-LTP, NHP control, WT TBS-LTP, and WT control) were collected 4 h after TBS stimulation, flash-frozen in liquid nitrogen, and stored at −80 °C. Total protein was extracted using TPER Tissue Protein Extraction Kit (Thermo Fisher Scientific) supplemented with Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific). Protein concentrations were determined by Bradford assay (Bio-Rad). Equal amounts of protein (20 μg per sample) were resolved by 10% SDS/PAGE and transferred to PVDF membranes. Membranes were incubated overnight at 4 °C with primary antibodies against CREB (1:500; Cell Signaling Technology) or BDNF (1:1,000; Abcam), followed by appropriate secondary antibodies. α-Tubulin (Sigma-Aldrich) was used as a loading control. Immunoreactive bands were visualized using SuperSignal West Pico Plus Chemiluminescent Substrate (Pierce Biotechnology) and quantified with ImageJ. Band intensities were normalized to the corresponding α-tubulin signal. For all experiments, slices obtained from a minimum of three independent biological samples (n= 3) were used for analysis.

Data Analysis and Statistics

In field electrophysiological recordings, the strength of synaptic responses was quantified by measuring the slope of the fEPSP (millivolts per millisecond). All data are presented as mean ± SEM. To assess statistical significance within groups, the Wilcoxon signed-rank test (denoted as Wilcox) was applied to compare the mean normalized fEPSP values at specific time points with the fEPSP at −15 minutes (baseline). For comparisons between different groups, the Mann-Whitney U test (denoted as U test) was used. Statistical significance was set at $p < 0.05$. Nonparametric tests were chosen because a Gaussian normal distribution could not always be assumed, especially given the small sample size and the analysis of prolonged recordings (37, 38). In vitro electrophysiology data are presented as "n," representing the number of slices.

For qRT-PCR and Western blot analyses, data from three to four independent experiments are presented as mean ± SD. Statistical significance was determined using Student's t test or one-way ANOVA, as appropriate. Differences were considered significant at $P < 0.05$. Analyses were performed using Prism (GraphPad Software).

Data availability

All behavioral, electrophysiological, immunohistochemical, and neurochemical data have been deposited at *https://osf.io* and are publicly available.

Acknowledgements

This work was supported by the NUHS Seed Fund (NUHSRO/2024/089/T1/Seed-Mar24/02) and the Ministry of Health (MOH-000641-00 and MOH-001883-00) awarded to S.S. C. L was supported by MOE-T2EP30121-0010.

Additional information

Author Contributions

Conceptualization: SS and CL; Methodology: AM,KK,YSC, WLW,SN, YPW,STW and SS; Investigation: AM,KK,YSC, WLW,SN; Visualization: AM,KK,YSC, WLW,SN; Funding acquisition: SS and CL; Project administration: SS and CL; Supervision: SS and CL; Writing – original draft: AM, SN and SS; Writing – review & editing: SN, WLW, CL and SS.

Funding

Funder	Grant reference number	Author
MOH | National Medical Research Council (NMRC)	MOH-001883-00	Sreedharan Sreedharan
Ministry of Education - Singapore (MOE)	MOE-T2EP30121-0010.	Camilo Libedinsky

Author ORCID iDs

Camilo Libedinsky: https://orcid.org/0000-0003-4980-4088

References

1. **Thome A,** et al. (2017) Evidence for an Evolutionarily Conserved Memory Coding Scheme in the Mammalian Hippocampus. *J Neurosci* **37**:2795-2801 https://doi.org/10.1523/jneurosci.3057-16.2017 | PubMed

2. **Martinez-Trujillo J** (2025) Why do primates have view cells instead of place cells?. *Trends in Cognitive Sciences* **29**:226-229 https://doi.org/10.1016/j.tics.2024.12.007 | PubMed

3. **Bin Ibrahim MZ,** Benoy A, Sajikumar S (2022) Long-term plasticity in the hippocampus: maintaining within and 'tagging'between synapses. *The FEBS journal* **289**:2176-2201 https://doi.org/10.1111/febs.16065 | PubMed
4. **Malenka RC,** Bear MF (2004) LTP and LTD: an embarrassment of riches. *Neuron* **44**:5-21 https://doi.org/10.1016/j.neuron.2004.09.012 | PubMed
5. **Urban NN,** Henze DA, Lewis DA, Barrionuevo G (1996) Properties of LTP induction in the CA3 region of the primate hippocampus. *Learning & Memory* **3**:86-95 https://doi.org/10.1101/lm.3.2-3.86 | PubMed
6. **Chen WR,** et al. (1996) Long-term modifications of synaptic efficacy in the human inferior and middle temporal cortex. *Proceedings of the National Academy of Sciences* **93**:8011-8015 https://doi.org/10.1073/pnas.93.15.8011 | PubMed
7. **Huang S,** et al. (2014) Associative Hebbian synaptic plasticity in primate visual cortex. *Journal of Neuroscience* **34**:7575-7579 https://doi.org/10.1523/jneurosci.0983-14.2014 | PubMed
8. **Seeman SC,** Mogen BJ, Fetz EE, Perlmutter SI (2017) Paired stimulation for spike-timing-dependent plasticity in primate sensorimotor cortex. *Journal of Neuroscience* **37**:1935-1949 https://doi.org/10.1523/jneurosci.2046-16.2017 | PubMed
9. **Blundon JA,** Zakharenko SS (2008) Dissecting the components of long-term potentiation. *Neuroscientist* **14**:598-608 https://doi.org/10.1177/1073858408320643 | PubMed
10. **Hernandez RV,** Navarro MM, Rodriguez WA, Martinez JL, LeBaron RG (2005) Differences in the magnitude of long-term potentiation produced by theta burst and high frequency stimulation protocols matched in stimulus number. *Brain Res Brain Res Protoc* **15**:6-13 https://doi.org/10.1016/j.brainresprot.2005.02.003 | PubMed
11. **Moore CI,** Browning MD, Rose GM (1993) Hippocampal plasticity induced by primed burst, but not long-term potentiation, stimulation is impaired in area CA1 of aged Fischer 344 rats. *Hippocampus* **3**:57-66 https://doi.org/10.1002/hipo.450030106 | PubMed
12. **Diamond DM,** Bennett MC, Fleshner M, Rose GM (1992) Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus* **2**:421-430 https://doi.org/10.1002/hipo.450020409 | PubMed
13. **Corradetti R,** Ballerini L, Pugliese AM, Pepeu G (1992) Serotonin blocks the long-term potentiation induced by primed burst stimulation in the CA1 region of rat hippocampal slices. *Neuroscience* **46**:511-518 https://doi.org/10.1016/0306-4522(92)90140-w | PubMed
14. **Pan B,** et al. (2011) Alterations of endocannabinoid signaling, synaptic plasticity, learning, and memory in monoacylglycerol lipase knock-out mice. *J Neurosci* **31**:13420-13430 https://doi.org/10.1523/jneurosci.2075-11.2011 | PubMed
15. **Costenla AR,** de Mendonça A, Ribeiro JA (1999) Adenosine modulates synaptic plasticity in hippocampal slices from aged rats. *Brain Res* **851**:228-234 https://doi.org/10.1016/s0006-8993(99)02194-0 | PubMed
16. **Sajikumar S,** Korte M (2011) Metaplasticity governs compartmentalization of synaptic tagging and capture through brain-derived neurotrophic factor (BDNF) and protein kinase Mzeta (PKMzeta). *Proc Natl Acad Sci U S A* **108**:2551-2556 https://doi.org/10.1073/pnas.1016849108 | PubMed
17. **Huang YY,** Kandel ER (2005) Theta frequency stimulation induces a local form of late phase LTP in the CA1 region of the hippocampus. *Learn Mem* **12**:587-593 https://doi.org/10.1101/lm.98905 | PubMed
18. **Frey U,** Morris RG (1997) Synaptic tagging and long-term potentiation. *Nature* **385**:533-536 https://doi.org/10.1038/385533a0 | PubMed
19. **Frey U,** Morris RG (1998) Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. *Trends in neurosciences* **21**:181-188 https://doi.org/10.1016/s0166-2236(97)01189-2 | PubMed

20. **Abraham WC** (2008) Metaplasticity: tuning synapses and networks for plasticity. *Nature Reviews Neuroscience* **9**:387-387 https://doi.org/10.1038/nrn2356 | PubMed

21. **Koopmans F,** et al. (2018) Comparative Hippocampal Synaptic Proteomes of Rodents and Primates: Differences in Neuroplasticity-Related Proteins. *Front Mol Neurosci* **11**:364 https://doi.org/10.3389/fnmol.2018.00364 | PubMed

22. **Navakkode S,** Sajikumar S, Sacktor TC, Frey JU (2010) Protein kinase Mzeta is essential for the induction and maintenance of dopamine-induced long-term potentiation in apical CA1 dendrites. *Learn Mem* **17**:605-611 https://doi.org/10.1101/lm.1991910 | PubMed

23. **Sajikumar S,** Navakkode S, Sacktor TC, Frey JU (2005) Synaptic tagging and cross-tagging: the role of protein kinase Mζ in maintaining long-term potentiation but not long-term depression. *Journal of Neuroscience* **25**:5750-5756 https://doi.org/10.1523/jneurosci.1104-05.2005 | PubMed

24. **Nguyen PV,** Kandel ER (1997) Brief theta-burst stimulation induces a transcription-dependent late phase of LTP requiring cAMP in area CA1 of the mouse hippocampus. *Learning & memory* **4**:230-243 https://doi.org/10.1101/lm.4.2.230 | PubMed

25. **Rivera DS,** et al. (2020) Effects of long-lasting social isolation and re-socialization on cognitive performance and brain activity: a longitudinal study in Octodon degus. *Scientific reports* **10**:18315 https://doi.org/10.1038/s41598-020-75026-4 | PubMed

26. **Chapman TR,** Barrientos RM, Ahrendsen JT, Maier SF, Patterson SL (2010) Synaptic correlates of increased cognitive vulnerability with aging: peripheral immune challenge and aging interact to disrupt theta-burst late-phase long-term potentiation in hippocampal area CA1. *Journal of Neuroscience* **30**:7598-7603 https://doi.org/10.1523/jneurosci.5172-09.2010 | PubMed

27. **Huang Y.-Y,** Kandel ER (2005) Theta frequency stimulation induces a local form of late phase LTP in the CA1 region of the hippocampus. *Learning & Memory* **12**:587-593 https://doi.org/10.1101/lm.98905 | PubMed

28. **Park AJ,** Shetty MS, Baraban JM, Abel T (2020) Selective role of the translin/trax RNase complex in hippocampal synaptic plasticity. *Molecular brain* **13**:145 https://doi.org/10.1186/s13041-020-00691-5 | PubMed

29. **Park P,** et al. (2019) On the role of calcium-permeable AMPARs in long-term potentiation and synaptic tagging in the rodent hippocampus. *Frontiers in synaptic neuroscience* **11**:4 https://doi.org/10.3389/fnsyn.2019.00004 | PubMed

30. **Glatigny M,** Geffroy F, van Kerkoerle T (2024) Comparison of the cortical hierarchy between macaque monkeys and mice based on cell-type specific microcircuits. *bioRxiv* 2024.2010. 2031.621258 https://doi.org/10.1101/2024.10.31.621258

31. **Krienen FM,** et al. (2020) Innovations present in the primate interneuron repertoire. *Nature* **586**:262-269 https://doi.org/10.1038/s41586-020-2781-z | PubMed

32. **Wildenberg GA,** et al. (2021) Primate neuronal connections are sparse in cortex as compared to mouse. *Cell Reports* **36** https://doi.org/10.1016/j.celrep.2021.109709 | PubMed

33. **Katzman A,** et al. (2021) Distinct transcriptomic profiles in the dorsal hippocampus and prelimbic cortex are transiently regulated following episodic learning. *Journal of Neuroscience* **41**:2601-2614 https://doi.org/10.1523/jneurosci.1557-20.2021 | PubMed

34. **Shimogori T,** Onishi K, Hoshino T, Nakanishi M (2024) Molecular architecture of primate specific neural circuit formation. *Research Square* rs.3.rs-4082064 https://doi.org/10.21203/rs.3.rs-4082064/v1 | PubMed

35. **Chong YS,** Ang SR, Sajikumar S (2025) Beyond boundaries: extended temporal flexibility in synaptic tagging and capture. *Communications Biology* **8**:553 https://doi.org/10.1038/s42003-025-07998-w | PubMed

36. **Navakkode S,** Sajikumar S, Frey JU (2004) The type IV-specific phosphodiesterase inhibitor rolipram and its effect on hippocampal long-term potentiation and synaptic tagging. *J Neurosci* **24**:7740-7744 https://doi.org/10.1523/jneurosci.1796-04.2004 | PubMed

37. **Pang KKL,** Sharma M, Krishna KK, Behnisch T, Sajikumar S (2019) Long-term population spike-timing-dependent plasticity promotes synaptic tagging but not cross-tagging in rat hippocampal area CA1. *Proc Natl Acad Sci U S A* **116**:5737-5746 https://doi.org/10.1073/pnas.1817643116 | PubMed

38. **Navakkode S,** et al. (2021) Sex-specific accelerated decay in time/activity-dependent plasticity and associative memory in an animal model of Alzheimer's disease. *Aging Cell* **20**:e13502 https://doi.org/10.1111/acel.13502 | PubMed

Peer reviews

Reviewer #1 (Public review):

Summary:

This is an important paper examining LTP induced by theta-burst stimulation in hippocampal slices from macaques and rats. While both species show theta-burst-late-LTP, only the non-human primate theta-burst-late-LTP showed synaptic tagging and capture that converts early-LTP into late-LTP in an independent synaptic pathway.

Strengths:

Synaptic tagging is a fundamental feature of repeated 100 Hz-tetanus-induced LTP, whereas theta-burst induction is arguably more physiologically relevant. Thus, synaptic tagging during theta-burst may differ in the two species, a distinction that may prove important in the mechanisms underlying the cognitive differences between the species.

Weaknesses:

Bursts repeated at the frequency (~5 Hz) of the endogenous theta rhythm induce strong LTP, primarily because this frequency disables feed-forward inhibition and allows sufficient postsynaptic depolarization to activate voltage-sensitive NMDA receptors. Therefore, the species differences may be due to differences in inhibition, rather than in molecular mechanisms of maintenance. One way to assess the relative strengths of this early induction mechanism in rats and macaques is to examine the "depolarization envelope" during the sequential bursts, which may be determined from the recordings already obtained. (Larson and Munkácsy, Theta-burst LTP, Brain Res 2015 Sep 24:1621:38-50. doi: 10.1016/j.brainres.2014.10.034)

Another issue is that the PKMzeta-antisense oligodeoxynucleotides block the synthesis of the kinase. However, Mei F, Nagappan G, Ke Y, Sacktor TC, Lu B (2011), BDNF Facilitates L-LTP Maintenance in the Absence of Protein Synthesis through PKMzeta. PLoS ONE 6(6):e21568, provided evidence that BDNF and theta-burst stimulation can act to increase PKMzeta by a protein synthesis-independent mechanism, presumably through decreased degradation. Therefore, the absence of an effect of the PKMzeta-antisense does not exclude the possibility that persistently increased PKMzeta is the mechanism of theta-burst-late-LTP maintenance in mice or macaques. This issue is worth discussing.

https://doi.org/10.7554/eLife.111886.1.sa3

Reviewer #2 (Public review):

Summary:

This study compares theta-burst stimulation (TBS)-induced synaptic plasticity in hippocampal CA1 slices from rats and non-human primates (Macaca fascicularis). The authors report that while TBS induces persistent LTP in both species, only primate hippocampal slices exhibit synaptic tagging and capture (STC) under these conditions. They further show increased

BDNF and PKMζ expression following TBS in primates and propose that a redundant BDNF/PKMζ signaling architecture supports persistent plasticity in primates, whereas rodent TBS-LTP depends primarily on BDNF. The work aims to identify species-specific specializations in associative plasticity with implications for translational neuroscience.

Strengths:

The topic is potentially important because direct comparisons of hippocampal plasticity mechanisms between rodents and primates are rare.

Weaknesses:

(1) Limited biological replication in the primate experiments

The manuscript's strongest claims rely on data obtained from 36 slices from 7 monkeys, qPCR analyses with n=3 biological replicates, and Western blot analyses with n=3 biological replicates. The effective sample size for species-level conclusions is therefore not large. The manuscript frequently treats slices as independent observations while drawing conclusions about species differences. This is particularly problematic for electrophysiological experiments because multiple slices appear to originate from the same animals. The statistical unit should be the animal, not the slice, unless nested analyses are performed.

The authors should (1) report the number of animals contributing to each experiment, (2) provide animal-level analyses, (3) use mixed-effects or hierarchical models where appropriate, and (4) clarify whether multiple slices from the same monkey contributed to the same experimental condition. Without these analyses, the evidence for species-specific mechanisms remains weaker than presented.

(2) The central STC conclusion requires stronger controls

The most important result is that TBS supports STC in primates but not rats (Figures 1F-G). However, several alternative explanations are not excluded. For example, only a single interval (30 min) between TBS and WTET is examined. Classical STC studies characterize tag duration, PRP availability window, and temporal asymmetry. The current work does not determine whether primates exhibit longer tag persistence, increased PRP synthesis, altered capture efficiency, or merely a shifted temporal window. A temporal series (e.g., {plus minus}15, {plus minus}30, {plus minus}60, {plus minus}90 min) would substantially strengthen the mechanistic interpretation.

(3) Species differences may reflect tissue quality or preparation differences

The manuscript compares 5-7 week-old rats with 5-7 year-old monkeys. These are very different developmental stages. Moreover, euthanasia methods, extraction procedures, and postmortem handling are different. These factors can affect BDNF expression, protein synthesis, LTP magnitude, and transcriptional responses. The authors should discuss these caveats more explicitly.

(4) Statistical reporting is incomplete

Many comparisons report exactly Wilcoxon p = 0.0313 and U-test p = 0.0022, across numerous experiments. This suggests very small sample sizes and discrete nonparametric distributions. The manuscript should report exact n values for each comparison, effect sizes, and confidence intervals.

Second, many genes and proteins are tested. No correction for multiple testing is described. The authors should state whether corrections were applied, and if not, justify this choice.

(5) Interpretation and significance

The study addresses an important and understudied question: whether associative synaptic plasticity mechanisms differ between rodents and primates. The finding that TBS can support STC in the primate hippocampus is potentially novel and impactful. However, the mechanistic evidence remains incomplete, the molecular analyses are underpowered, and several key controls are missing. At present, the data support the conclusion that under the specific experimental conditions tested, TBS-induced plasticity in primate hippocampal slices exhibits greater associative persistence than in rat slices.

The stronger claims regarding evolutionary specialization, fundamentally distinct plasticity rules, altered STC thresholds, and redundant BDNF/PKMζ architecture require additional experimental support.

https://doi.org/10.7554/eLife.111886.1.sa2

Reviewer #3 (Public review):

Summary:

In this manuscript, the authors have undertaken an investigation of differences between two mammalian species, the brown rat and the crab-eating macaque, in the mechanisms supporting a well-established model of long-term Hebbian synaptic plasticity, Schaffer collateral to CA1 Long-term potentiation (LTP) in the hippocampus. LTP has been long-studied and deeply characterised due to its potential importance in modeling a strong candidate process for the central mechanism of learning and memory. LTP was first discovered in lagomorphs (rabbits), but has since been much more widely studied in rodents (mostly rats and mice), and there has been some complementary work revealing LTP in non-human primates and even in humans, revealing largely overlapping canonical mechanisms of induction, expression, and maintenance. More specifically, this study puts a particular focus on the fascinating associative features of this form of lasting synapse-specific modification, in which a synaptic input can be stimulated with a relatively weak induction protocol that will not produce lasting plasticity on its own, but can undergo lasting LTP if paired with stronger stimulation on a separate synaptic input to the same neuron. This associativity mechanism is particularly attractive within the Hebbian synaptic plasticity framework as it provides a candidate mechanism for associative forms of learning in which stimulus-stimulus, stimulus-reward, stimulus-punishment, or action-outcome associations are formed. A particularly attractive feature of this associative LTP is that there can also be a substantial time-lag between the strong stimulation of one pathway and the weaker stimulation of the other synaptic input, which only undergoes lasting LTP by hijacking the proteins synthesized as a result of strong stimulation elsewhere. This observation has led to the famous tagging and capture hypothesis as an explanation of how such synapse-specific change can be achieved on both stimulated inputs but not on other synaptic inputs, given the potential requirement for cell-wide protein synthesis. This theory, for which there is very strong experimental evidence, posits that a protein tag is left at synapses that have been stimulated with sufficient vigor in recent history, serving as a key mechanism to ensure that those weakly stimulated synapses will undergo change when a larger-scale LTP event occurs due to stronger stimulation elsewhere within a relevant time window. Again, this idea is attractive as it can explain how we might form associations between events that occur slightly separated in time. The manuscript goes on to show that an induction protocol that is particularly physiologically relevant, theta burst stimulation, produces this tag and capture associative effect in ex vivo slices of Macaque hippocampus, much more readily than in side-by-side ex vivo slices of rat hippocampus. Moreover, the manuscript delves into the importance of well-characterised LTP maintenance mechanisms, including PKMzeta and BDNF, which are key factors that ensure that altered synaptic change is maintained for long periods of time despite substantial molecular turnover in the neuron. The observation in this manuscript is that a degree of redundancy for these mechanisms exists in the primate species but not the rodent species, as

both mechanisms need to be inhibited to return LTP to baseline in the Macaque, but only one needs to be inhibited to have that effect in the rat. A major emphasis of this study is that there may be a step-wise difference in associative learning mechanisms between rodents and primates that may contribute to their differing cognitive capacities, although I believe a lot more evidence would be required to reach that conclusion.

Strengths:

The strengths of this study are that it is technically very proficient and is from a laboratory that has a long history of seminal work on synaptic tagging and capture. The cross-species comparison, particularly involving non-human primates, is also very hard to achieve, and a major strength here is the side-by-side comparison of slices from rat and monkeys. Further strengths of the study are the use of a number of experimental strategies, including both observation and intervention, to demonstrate differential involvement of LTP maintenance mechanisms. A final major strength is conceptual, as it is undoubtedly useful not only to identify shared mechanisms of plasticity between commonly used model organisms and either humans or much more closely related species such as old world monkeys, but also to reveal differences that have the potential to contribute to differences in memory/cognition.

Weaknesses:

The findings of this study are a very useful building block for understanding how generalisable mechanisms of LTP are. However, arriving at really substantial conclusions from these findings is challenging, as there are a number of variables that are unaccounted for in this study that may explain the differences that have been observed between rats and monkeys. One example of a potential confound to these interpretations is that rats are nocturnal/crepuscular animals, and macaques are diurnal animals. Thus, to undertake a like-for-like comparison, it would be necessary for the rats to be on a reversed light-dark cycle to ensure that the wake cycle of the rat (dark) is being compared with the wake cycle of the monkey (light). It is possible that the authors have done this, but it is not mentioned in the methods section. The reason this is important is that there is a substantial body of work indicating that different mechanisms are at play in hippocampal LTP during wake and sleep. Transcripts and proteins related to synaptic function are dramatically differentially regulated during sleep-wake cycles, and phosphorylation states of key proteins involved in plasticity are also altered. Moreover, synaptic tagging and capture are specifically disrupted by sleep deprivation. Perhaps the authors have already considered this factor and appropriately reversed the light-dark cycle of their rat subjects, in which case a clarification in the manuscript would be useful. Nevertheless, I have used this as an example because there is a variety of potential confounds that may explain the difference between SC-CA1 TBS LTP in rats and monkeys, e.g., circadian rhythms, degree of enrichment, natural light vs indoor lighting, diet, degree of inbreeding, strain, etc. Thus, to make strong conclusions about the potential for differences in plasticity rules/mechanisms and how those may contribute to differences in cognition, I think it would be necessary to compare a wider variety of species, including a good representation of each order (e.g., nocturnal rats and diurnal squirrels, new and old world primates) and not just a single exemplar. I understand, of course, that this is really pushing the boundaries of practicality, but I see no other way to make a strong conclusion or to generalise to mechanisms or properties of plasticity in rodents vs primates. Thus, while I believe the manuscript presents really admirable work, I am not sure the findings are at all easy to interpret.

https://doi.org/10.7554/eLife.111886.1.sa1

Author response:

> ***eLife Assessment***

> *This is a potentially important study comparing LTP mechanisms between primates and rodents. The experimental methods have some possible confounds, and the power (replicates) and design of the statistical methods could be strengthened, hence the support for the central claims of species differences is currently incomplete.*

We thank the Editor and the Reviewers for taking the time to carefully review our manuscript and for providing constructive comments and suggestions, as well as the opportunity to revise our work.

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *This is an important paper examining LTP induced by theta-burst stimulation in hippocampal slices from macaques and rats. While both species show theta-burst-late-LTP, only the non-human primate theta-burst-late-LTP showed synaptic tagging and capture that converts early-LTP into late-LTP in an independent synaptic pathway.*
>
> *Strengths:*
>
> *Synaptic tagging is a fundamental feature of repeated 100 Hz-tetanus-induced LTP, whereas theta-burst induction is arguably more physiologically relevant. Thus, synaptic tagging during theta-burst may differ in the two species, a distinction that may prove important in the mechanisms underlying the cognitive differences between the species.*
>
> *Weaknesses:*
>
> *Bursts repeated at the frequency (~5 Hz) of the endogenous theta rhythm induce strong LTP, primarily because this frequency disables feed-forward inhibition and allows sufficient postsynaptic depolarization to activate voltage-sensitive NMDA receptors. Therefore, the species differences may be due to differences in inhibition, rather than in molecular mechanisms of maintenance. One way to assess the relative strengths of this early induction mechanism in rats and macaques is to examine the "depolarization envelope" during the sequential bursts, which may be determined from the recordings already obtained. (Larson and Munkácsy, Theta-burst LTP, Brain Res 2015 Sep 24:1621:38-50. doi: 10.1016/j.brainres.2014.10.034)*
>
> *Another issue is that the PKMzeta-antisense oligodeoxynucleotides block the synthesis of the kinase. However, Mei F, Nagappan G, Ke Y, Sacktor TC, Lu B (2011), BDNF Facilitates L-LTP Maintenance in the Absence of Protein Synthesis through PKMzeta. PLoS ONE 6(6):e21568, provided evidence that BDNF and theta-burst stimulation can act to increase PKMzeta by a protein synthesis-independent mechanism, presumably through decreased degradation. Therefore, the absence of an effect of the PKMzeta-antisense does not exclude the possibility that persistently increased PKMzeta is the mechanism of theta-burst-late-LTP maintenance in mice or macaques. This issue is worth discussing.*

We sincerely thank the reviewer for the positive evaluation of our study and for highlighting the significance of examining synaptic tagging and capture following theta-burst stimulation (TBS) in rodents and non-human primates.

We agree that TBS is a physiologically relevant induction paradigm and that differences in inhibitory circuit dynamics may also contribute to the species-specific effects observed in our study. As highlighted by Larson and Munkácsy (2015), repeated bursts delivered at theta frequency (~5 Hz) can transiently suppress feed-forward inhibition through GABAB receptor-mediated mechanisms, thereby enhancing postsynaptic depolarization and facilitating NMDA

receptor activation. We therefore agree that species differences in inhibitory regulation and burst-evoked depolarization may contribute to the distinct expression of synaptic tagging and capture observed between rats and non-human primates.

We further agree that analysis of the "depolarization envelope" during sequential bursts may provide additional insight into the relative strengths of early induction mechanisms. We will therefore perform these analyses using the existing recordings and compare the depolarization envelope between rodents and NHPs in the revised manuscript. Following the reviewer's suggestion, we will expand the Discussion section to acknowledge the potential contribution of inhibitory circuit dynamics and depolarization envelope differences during sequential bursts.

Importantly, however, we believe that differences in downstream molecular maintenance mechanisms also contribute to these species-specific effects. In support of this, our molecular analyses revealed enhanced recruitment of plasticity-related proteins and transcriptional pathways in NHP hippocampus following TBS, including increased expression of BDNF and PKCζ. These findings suggest that both induction-related network properties and downstream molecular stabilization mechanisms may collectively contribute to the enhanced associative plasticity observed in NHPs.

We also thank the reviewer for the important point regarding PKMζ antisense experiments and the study by Mei et al. (2011). We agree that the absence of an effect of PKMζ antisense oligodeoxynucleotides does not necessarily exclude a role for persistently elevated PKMζ in the maintenance of theta-burst late-LTP. As demonstrated by Mei et al., BDNF together with theta-burst stimulation can maintain late-LTP in the absence of protein synthesis, potentially through stabilization of PKMζ protein levels by reducing degradation rather than through de novo synthesis. However, these findings are not directly comparable to our study, since our experiments involved theta-burst stimulation alone without exogenous BDNF application. Interestingly, our results suggest species-specific differences in the interaction between BDNF and PKMζ signaling pathways. In rats, TrkB/Fc-mediated blockade of BDNF impaired TBS-LTP maintenance, whereas PKMζ inhibition alone had no significant effect. In contrast, in NHP hippocampal slices, inhibition of either BDNF signaling or PKMζ alone failed to abolish late-LTP, whereas simultaneous inhibition of both pathways disrupted LTP maintenance.

These findings suggest that endogenous BDNF signaling and PKMζ may operate through partially redundant or compensatory mechanisms, particularly in the primate hippocampus. Therefore, although our findings indicate that de novo PKMζ synthesis may not be strictly required under the present experimental conditions, we cannot fully exclude the possibility that protein synthesis-independent stabilization or maintenance of PKMζ contributes to theta-burst late-LTP maintenance in rodents or NHPs. We will now clarify this point in the revised Discussion section.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *This study compares theta-burst stimulation (TBS)-induced synaptic plasticity in hippocampal CA1 slices from rats and non-human primates (Macaca fascicularis). The authors report that while TBS induces persistent LTP in both species, only primate hippocampal slices exhibit synaptic tagging and capture (STC) under these conditions. They further show increased BDNF and PKMζ expression following TBS in primates and propose that a redundant BDNF/PKMζ signaling architecture supports persistent plasticity in primates, whereas rodent TBS-LTP depends primarily on BDNF. The work aims to identify species-specific specializations in associative plasticity with implications for translational neuroscience.*

> *Strengths:*
>
> *The topic is potentially important because direct comparisons of hippocampal plasticity mechanisms between rodents and primates are rare.*
>
> *Weaknesses:*
>
> *(1) Limited biological replication in the primate experiments*
>
> *The manuscript's strongest claims rely on data obtained from 36 slices from 7 monkeys, qPCR analyses with n=3 biological replicates, and Western blot analyses with n=3 biological replicates. The effective sample size for species-level conclusions is therefore not large. The manuscript frequently treats slices as independent observations while drawing conclusions about species differences. This is particularly problematic for electrophysiological experiments because multiple slices appear to originate from the same animals. The statistical unit should be the animal, not the slice, unless nested analyses are performed.*
>
> *The authors should (1) report the number of animals contributing to each experiment, (2) provide animal-level analyses, (3) use mixed-effects or hierarchical models where appropriate, and (4) clarify whether multiple slices from the same monkey contributed to the same experimental condition. Without these analyses, the evidence for species-specific mechanisms remains weaker than presented.*

We thank the reviewer for this important and thoughtful comment regarding statistical interpretation and biological replication. We agree that, particularly for electrophysiological experiments where multiple slices may originate from the same animal, the effective sample size for species-level conclusions should be considered at the animal level rather than solely at the slice level.

In the revised manuscript, we will clearly indicate the number of biological replicates (animals) together with the number of slices contributing to each electrophysiological experiment, as well as the biological replicates used for qPCR and Western blot analyses. We will also clarify whether multiple slices from the same NHP/rat contributed to the same experimental condition. These details will be incorporated into the figures and figure legends wherever appropriate.

In addition, we will perform animal-level analyses by averaging slice responses within each animal prior to statistical comparison and, where appropriate, apply hierarchical or mixed-effects statistical models to account for the nested structure of slices within animals.

We acknowledge that the number of non-human primates (NHPs) available for this study was inherently limited because of the substantial ethical, logistical, financial, and technical challenges associated with primate electrophysiology and tissue collection. Consequently, achieving sample sizes comparable to rodent studies is often not feasible in NHP research. Nevertheless, to further strengthen the biological robustness of the findings, we are currently in the process of obtaining additional NHP brain samples and plan to repeat key experiments in an additional 3-4 animals. We believe these revisions and additional experiments will substantially strengthen the statistical rigor and overall interpretation of the study.

> *(2) The central STC conclusion requires stronger controls*
>
> *The most important result is that TBS supports STC in primates but not rats (Figures 1F-G). However, several alternative explanations are not excluded. For example, only a single interval (30 min) between TBS and WTET is examined. Classical STC studies characterize tag duration, PRP availability window, and temporal asymmetry. The current work does not determine whether primates exhibit longer tag persistence,*

> *increased PRP synthesis, altered capture efficiency, or merely a shifted temporal window. A temporal series (e.g., {plus minus}15, {plus minus}30, {plus minus}60, {plus minus}90 min) would substantially strengthen the mechanistic interpretation.*

We thank the reviewer for this insightful comment regarding the mechanistic interpretation of the STC findings. In the present study, we selected the 30 min interval based on well-established classical STC paradigms in rodents, where this interval reliably falls within the effective tagging and capture window. Using this experimentally validated interval allowed us to directly compare whether TBS is sufficient to support STC in primates versus rats under equivalent experimental conditions. Accordingly, the primary objective of this study was to determine whether TBS-induced STC varies across species, rather than to comprehensively define the temporal dynamics of the tagging window.

We agree, however, that the current experiments do not distinguish whether the primate-specific effect reflects prolonged tag persistence, enhanced plasticity-related protein (PRP) synthesis, altered capture efficiency, or a shifted temporal window. Addressing these possibilities would indeed require systematic temporal interval analyses (e.g., ±15, ±30, ±60, and ±90 min), which represent important future directions. Such experiments are particularly challenging in non-human primates because the availability of primate tissue and experimental resources for large-scale electrophysiological studies remains limited and is currently beyond our experimental capacity due to substantial ethical, logistical, financial, and technical constraints.

Nevertheless, we fully agree with the reviewer that these experiments are important for advancing the mechanistic interpretation of the findings. Similar temporal analyses have recently proven informative in our rodent studies (Chong YS, Ang SR, Sajikumar S. Commun Biol. 2025;8:553). Importantly, we are currently in the process of obtaining additional non-human primate samples and plan to extend the present work by examining an additional 60 min temporal interval to further characterize the temporal properties of synaptic tagging and capture in non-human primates.

> *(3) Species differences may reflect tissue quality or preparation differences*
>
> *The manuscript compares 5-7 week-old rats with 5-7 year-old monkeys. These are very different developmental stages. Moreover, euthanasia methods, extraction procedures, and post-mortem handling are different. These factors can affect BDNF expression, protein synthesis, LTP magnitude, and transcriptional responses. The authors should discuss these caveats more explicitly.*

We thank the reviewer for raising this important and insightful point. We agree that differences in developmental stage between the experimental groups represent an important consideration when interpreting potential species-dependent effects. In the present study, rat experiments were performed in 5-7 week-old animals, whereas non-human primate (NHP) tissues were obtained from 5-7-year-old monkeys. This difference largely reflects the practical, ethical, and logistical constraints associated with NHP research and tissue availability. We acknowledge that these ages are not developmentally equivalent and that maturation state may influence BDNF signaling, protein synthesis capacity, synaptic plasticity thresholds, and transcriptional responses relevant to late-LTP and STC mechanisms.

We also recognize that differences in euthanasia procedures, tissue extraction, slice preparation, and postmortem handling between rodent and primate tissues may influence tissue physiology and electrophysiological properties. Although extensive care was taken to optimize tissue viability and maintain stable recordings within each species, these variables cannot be completely excluded as contributing factors to the observed differences.

Accordingly, we will revise the Discussion section to more explicitly acknowledge these

limitations and clarify that our findings support potential species-dependent differences under the present experimental conditions, rather than definitive intrinsic species-specific mechanisms. Nevertheless, despite the inherent challenges associated with NHP electrophysiological studies, we believe that the present findings provide an important initial framework for understanding the translational relevance of synaptic tagging and capture mechanisms across species.

> *(4) Statistical reporting is incomplete*
>
> *Many comparisons report exactly Wilcoxon p = 0.0313 and U-test p = 0.0022, across numerous experiments. This suggests very small sample sizes and discrete nonparametric distributions. The manuscript should report exact n values for each comparison, effect sizes, and confidence intervals.*
>
> *Second, many genes and proteins are tested. No correction for multiple testing is described. The authors should state whether corrections were applied, and if not, justify this choice.*

We thank the reviewer for this important comment regarding statistical reporting and interpretation. We agree that the repeated occurrence of identical exact p-values in several nonparametric analyses reflects the relatively small sample sizes and the discrete nature of the statistical distributions. This issue is particularly relevant for the NHP experiments, where biological replication is inherently limited because of the substantial ethical, logistical, financial, and technical challenges associated with obtaining and processing primate tissue.

In the revised manuscript, we will provide exact n values for all comparisons, including the number of biological replicates (animals) and slices where applicable. We will also include additional statistical details, including effect sizes and confidence intervals where appropriate, to improve transparency and facilitate interpretation of the reported findings. Furthermore, we are currently in the process of obtaining additional NHP samples and will attempt to include more biological replicates in the revised version to further strengthen the robustness of the analyses.

We also agree that the issue of multiple testing should be addressed more explicitly, particularly because multiple genes and proteins were examined. In the revised manuscript, we will clearly state the statistical correction methods applied for multiple comparisons where appropriate. For analyses in which corrections were not applied, we will provide justification, noting that several experiments were based on hypothesis-driven candidate targets rather than exploratory large-scale screening analyses. These statistical considerations will be clarified in the Methods and Results sections.

> *(5) Interpretation and significance*
>
> *The study addresses an important and understudied question: whether associative synaptic plasticity mechanisms differ between rodents and primates. The finding that TBS can support STC in the primate hippocampus is potentially novel and impactful. However, the mechanistic evidence remains incomplete, the molecular analyses are underpowered, and several key controls are missing. At present, the data support the conclusion that under the specific experimental conditions tested, TBS-induced plasticity in primate hippocampal slices exhibits greater associative persistence than in rat slices.*
>
> *The stronger claims regarding evolutionary specialization, fundamentally distinct plasticity rules, altered STC thresholds, and redundant BDNF/PKMζ architecture require additional experimental support.*

We thank the reviewer for this thoughtful and balanced assessment of our work. We agree that the present data primarily support the conclusion that, under the specific experimental

conditions examined, TBS-induced plasticity in primate hippocampal slices exhibits greater associative persistence than that observed in rat slices. We also agree that broader interpretations regarding evolutionary specialization, fundamentally distinct plasticity rules, altered STC thresholds, and potentially redundant BDNF/PKMζ-related mechanisms require additional mechanistic investigation and experimental validation.

Accordingly, we will moderate these interpretations throughout the revised manuscript and clearly state that these conclusions remain preliminary. We will further emphasize that additional experiments, including increased biological replication, expanded temporal analyses, and further mechanistic investigations, will be necessary to more conclusively define the basis of the observed species-dependent differences. Within our current experimental capacity, we are actively working to obtain additional non-human primate samples and plan to incorporate additional biological replicates and key follow-up experiments in the revised version to further strengthen the robustness of the findings.

At the same time, we believe the present study provides an important initial contribution to an understudied area by directly examining synaptic tagging and capture mechanisms in the primate hippocampus. Given the limited availability of non-human primate electrophysiological data in the field, these findings may offer a valuable framework for future studies investigating the translational and evolutionary relevance of associative synaptic plasticity mechanisms across species.

> ***Reviewer #3 (Public review):***
>
> *Summary:*
>
> *In this manuscript, the authors have undertaken an investigation of differences between two mammalian species, the brown rat and the crab-eating macaque, in the mechanisms supporting a well-established model of long-term Hebbian synaptic plasticity, Schaffer collateral to CA1 Long-term potentiation (LTP) in the hippocampus. LTP has been long-studied and deeply characterised due to its potential importance in modeling a strong candidate process for the central mechanism of learning and memory. LTP was first discovered in lagomorphs (rabbits), but has since been much more widely studied in rodents (mostly rats and mice), and there has been some complementary work revealing LTP in non-human primates and even in humans, revealing largely overlapping canonical mechanisms of induction, expression, and maintenance. More specifically, this study puts a particular focus on the fascinating associative features of this form of lasting synapse-specific modification, in which a synaptic input can be stimulated with a relatively weak induction protocol that will not produce lasting plasticity on its own, but can undergo lasting LTP if paired with stronger stimulation on a separate synaptic input to the same neuron. This associativity mechanism is particularly attractive within the Hebbian synaptic plasticity framework as it provides a candidate mechanism for associative forms of learning in which stimulus-stimulus, stimulus-reward, stimulus-punishment, or action-outcome associations are formed. A particularly attractive feature of this associative LTP is that there can also be a substantial time-lag between the strong stimulation of one pathway and the weaker stimulation of the other synaptic input, which only undergoes lasting LTP by hijacking the proteins synthesized as a result of strong stimulation elsewhere. This observation has led to the famous tagging and capture hypothesis as an explanation of how such synapse-specific change can be achieved on both stimulated inputs but not on other synaptic inputs, given the potential requirement for cell-wide protein synthesis. This theory, for which there is very strong experimental evidence, posits that a protein tag is left at synapses that have been stimulated with sufficient vigor in recent history, serving as a key mechanism to ensure that those weakly stimulated synapses will undergo change when a larger-scale LTP event occurs due to stronger stimulation elsewhere within a relevant time window. Again, this idea is attractive as it can explain how we might form associations between events that occur*

slightly separated in time. The manuscript goes on to show that an induction protocol that is particularly physiologically relevant, theta burst stimulation, produces this tag and capture associative effect in ex vivo slices of Macaque hippocampus, much more readily than in side-by-side ex vivo slices of rat hippocampus. Moreover, the manuscript delves into the importance of well-characterised LTP maintenance mechanisms, including PKMzeta and BDNF, which are key factors that ensure that altered synaptic change is maintained for long periods of time despite substantial molecular turnover in the neuron. The observation in this manuscript is that a degree of redundancy for these mechanisms exists in the primate species but not the rodent species, as both mechanisms need to be inhibited to return LTP to baseline in the Macaque, but only one needs to be inhibited to have that effect in the rat. A major emphasis of this study is that there may be a step-wise difference in associative learning mechanisms between rodents and primates that may contribute to their differing cognitive capacities, although I believe a lot more evidence would be required to reach that conclusion.

Strengths:

The strengths of this study are that it is technically very proficient and is from a laboratory that has a long history of seminal work on synaptic tagging and capture. The cross-species comparison, particularly involving non-human primates, is also very hard to achieve, and a major strength here is the side-by-side comparison of slices from rat and monkeys. Further strengths of the study are the use of a number of experimental strategies, including both observation and intervention, to demonstrate differential involvement of LTP maintenance mechanisms. A final major strength is conceptual, as it is undoubtedly useful not only to identify shared mechanisms of plasticity between commonly used model organisms and either humans or much more closely related species such as old world monkeys, but also to reveal differences that have the potential to contribute to differences in memory/cognition.

Weaknesses:

The findings of this study are a very useful building block for understanding how generalisable mechanisms of LTP are. However, arriving at really substantial conclusions from these findings is challenging, as there are a number of variables that are unaccounted for in this study that may explain the differences that have been observed between rats and monkeys. One example of a potential confound to these interpretations is that rats are nocturnal/crepuscular animals, and macaques are diurnal animals. Thus, to undertake a like-for-like comparison, it would be necessary for the rats to be on a reversed light-dark cycle to ensure that the wake cycle of the rat (dark) is being compared with the wake cycle of the monkey (light). It is possible that the authors have done this, but it is not mentioned in the methods section. The reason this is important is that there is a substantial body of work indicating that different mechanisms are at play in hippocampal LTP during wake and sleep. Transcripts and proteins related to synaptic function are dramatically differentially regulated during sleep-wake cycles, and phosphorylation states of key proteins involved in plasticity are also altered. Moreover, synaptic tagging and capture are specifically disrupted by sleep deprivation. Perhaps the authors have already considered this factor and appropriately reversed the light-dark cycle of their rat subjects, in which case a clarification in the manuscript would be useful. Nevertheless, I have used this as an example because there is a variety of potential confounds that may explain the difference between SC-CA1 TBS LTP in rats and monkeys, e.g., circadian rhythms, degree of enrichment, natural light vs indoor lighting, diet, degree of inbreeding, strain, etc. Thus, to make strong conclusions about the potential for differences in plasticity rules/mechanisms and how those may contribute to differences in cognition, I think it would be necessary to compare a wider variety of species, including a good representation of each order (e.g., nocturnal rats and diurnal

> *squirrels, new and old world primates) and not just a single exemplar. I understand, of course, that this is really pushing the boundaries of practicality, but I see no other way to make a strong conclusion or to generalise to mechanisms or properties of plasticity in rodent's vs primates. Thus, while I believe the manuscript presents really admirable work, I am not sure the findings are at all easy to interpret.*

We thank the reviewer for this thoughtful and insightful comment, as well as for the encouraging appreciation of our long-duration plasticity recordings and associative plasticity experiments, which are both technically demanding and time-intensive. We fully agree that interpretation of cross-species differences in synaptic plasticity requires careful consideration of multiple biological and environmental variables, including circadian state, enrichment conditions, strain differences, diet, lighting conditions, and species-specific behavioral ecology.

Regarding the specific concern related to circadian phase and sleep-wake state, the reviewer raises an important point. Rats are nocturnal animals, whereas macaques are diurnal, and hippocampal plasticity mechanisms are known to be influenced by circadian rhythms and sleep-dependent regulation of synaptic proteins and signaling pathways. Previous studies have demonstrated modulation of LTP, synaptic tagging and capture and protein synthesis in rats across normal sleep-wake cycles. We therefore agree that these factors may influence plasticity outcomes and should be carefully considered in comparative studies.

Studies have further shown that theta frequency is highly sensitive to sleep-related manipulations. Specifically, theta frequency decreases immediately after sleep, remains elevated during sleep deprivation, and rapidly declines following recovery sleep. In aged animals, these effects appear comparatively attenuated, suggesting reduced sleep-dependent modulation of theta dynamics with aging. Therefore, disruption of normal circadian or sleep-wake patterns may significantly alter theta activity and associated plasticity mechanisms within a species and may not accurately reflect physiological baseline states (Utku Kaya et al., 2026).

In our experiments, recordings from rats and macaques were performed during their respective active phases under standardized laboratory housing conditions, and we will further clarify these details in the revised Methods section. Nevertheless, we acknowledge that circadian state and related physiological variables cannot be completely excluded as contributing factors to the observed differences between species.

More broadly, we agree with the reviewer that the present study does not permit definitive conclusions regarding universal "rodent versus primate" rules of synaptic plasticity. Our intention was not to propose a generalized dichotomy between rodents and primates, but rather to report that, under the experimental conditions used here, SC-CA1 TBS-LTP and associated synaptic tagging mechanisms differed between rats and macaques. We agree that broader evolutionary or cognitive interpretations would require systematic comparative analyses across multiple species, including both nocturnal and diurnal rodents as well as diverse primate species. Such studies would provide a stronger framework for distinguishing conserved versus species-specific mechanisms of plasticity.

At the same time, we believe the present findings remain important because they provide one of the first direct experimental comparisons of SC-CA1 TBS-LTP-associated plasticity mechanisms between rodents and non-human primates under controlled ex vivo conditions. Although the interpretation should be done cautiously, the observed differences raise the possibility that certain metaplastic or protein synthesis-dependent mechanisms may not be fully conserved across species. Accordingly, we will revise the Discussion section to better emphasize the exploratory and comparative nature of the study, while explicitly acknowledging the limitations and potential confounding factors highlighted by the reviewer.

https://doi.org/10.7554/eLife.111886.1.sa0