Unit: Cellular & Molecular Synaptic Function Unit
Principal Investigator: Tomoyuki Takahashi
Research Theme: Regulatory mechanisms of transmitter release

Abstract

This is the final FY for this unit before closure owing to the retirement of the PI Tomoyuki Takahashi. Main studies completed within this FY are three-fold; first one is on the release site clearance physiology at the calyx of Held and hippocampal synapses in rodent brain slices by Satyajit Mahapatra and Tomoyuki Takahashi published in ***eLife***. The second study is on the mechanism of anoxia-induced LTP in mice hippocampal slices by Han Ying Wang et al published in ***iScience***. The third study is on the rescuing effect of the dynamin-microtubule binding inhibitor peptide PHDP5 on the learning/memory deficits of Alzheimer's disease (AD) model mice by Anna Chang et al. published in ***Brain Research***. Among these products, the first one is upon physiological mechanisms underlying synaptic transmission and other two studies are upon mechanisms of synaptic dysfunctions. Synapses play pivotal roles in regulating a variety of brain functions. Such synaptic activities are supported and regulated by synaptic molecules and their mutual interactions. Once homeostatic molecular balance is distorted, neurological symptoms such as motor, sensory or cognitive dysfunctions arise depending upon intrinsic physiological roles of synapses involved. Thus, it is fundamental to identify the primary molecular/cellular target impaired by pathogenic molecules. From a clinical point of view, the last two papers have provided therapeutic platforms on which specific and effective reagents for rescuing symptoms of human neurological diseases, such as AD or brain ischemia, can be developed.

1. Staffs

- Dr. Tetsuya Hori, Group Leader
- Dr. Zacharie Taoufiq, Staff Scientist
- Dr. Satyajit Mahapatra, Staff Scientist
- Dr. Anna Chia-Jung Chang, Postdoctoral Scholar
- Dr. Dimitar Dimitrov, Technical Staff (until August, 2023)
- Dr. Patrick Stoney, Technical Staff
- Dr. Marina Khandarkhaeva, Technical Staff
- Ms. Saori Araki, Research Assistant (part time)
- Ms. Asmaa Yahia, Research Assistant (part time)
- Ms. Yuka Matsumura, Research Intern (from July to October, 2023)
- Ms. Sayori Gordon, Research Unit Administrator

2. Collaborations

2.1 Functional and proteomics analyses of psychiatric diseases at patient's iPSC-derived synapses

- Name of partner organization**:** University of the Ryukyus
- Type of partnership: Scientific Collaboration
- Researchers:
    - Name of principal researcher: Dr. Masayuki Matsushita
    - Name of researcher: Dr. Gakuya Takamatsu

2.2 Identification of MT-dynamin binding domain

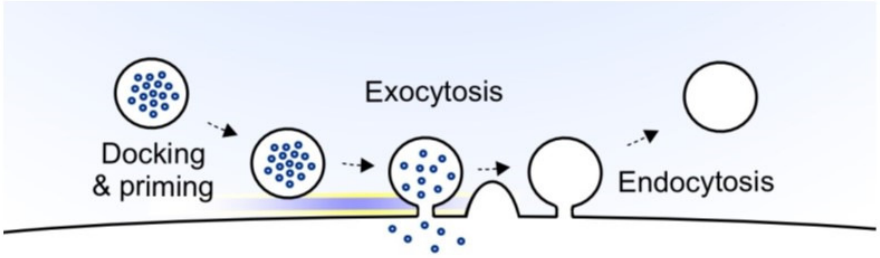
- Name of partner organization: Okayama University
- Type of partnership: Scientific Collaboration
- Researchers:
    - Name of principal researcher: Dr. Kohji Takei
    - Name of researcher: Dr. Hiroshi Yamada

2.3 Presynaptic functional roles of brevican

- Name of partner organization: Kyoto University
- Type of partnership: Scientific Collaboration
- Researchers:
    - Name of principal researcher: Dr. Takayasu Higo
    - Name of researcher: Dr. Takayasu Higo

3. Activities and Findings

3.1 **Physiological roles of endocytosis and presynaptic scaffold in vesicle replenishment at fast and slow central synapses** Satyajit Mahapatra and Tomoyuki Takahashi (*eLife* 2023).



Chemical synaptic transmission depends upon fusion of transmitter-filled vesicles with the presynaptic membrane. At presynaptic terminals, there are a limited number of vesicular release sites, which can become refractory while discharged vesicles are remaining at the site, inhibiting subsequent vesicle fusion. Endocytic and scaffold proteins are thought to underlie this site-clearance mechanism. However, the physiological significance of this mechanism at diverse mammalian central synapses remains unknown. We tested this in a physiologically optimized condition using action potential evoked EPSCs at fast calyx synapse and relatively slow hippocampal CA1 synapse, in post-hearing mice brain slices at 37°C and in 1.3 mM [Ca^{2+}] (optimally physiological condition).

Pharmacological block of endocytosis using Dynasore or Pitstop-2 enhanced synaptic depression at the calyx synapse during repetitive stimulations at 100 Hz but not significantly at 10 Hz (Fig 1), whereas it attenuated synaptic facilitation during 25 Hz stimulation at the hippocampal synapse (Fig 2).

Fig 1 EPSCs at the calyx of Held evoked by a train of fiber stimulations.

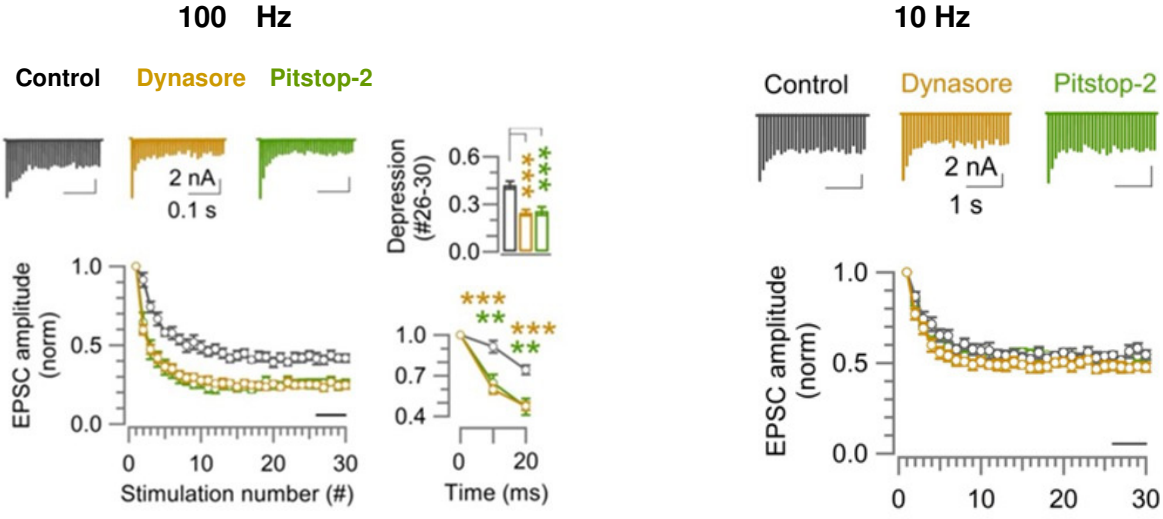
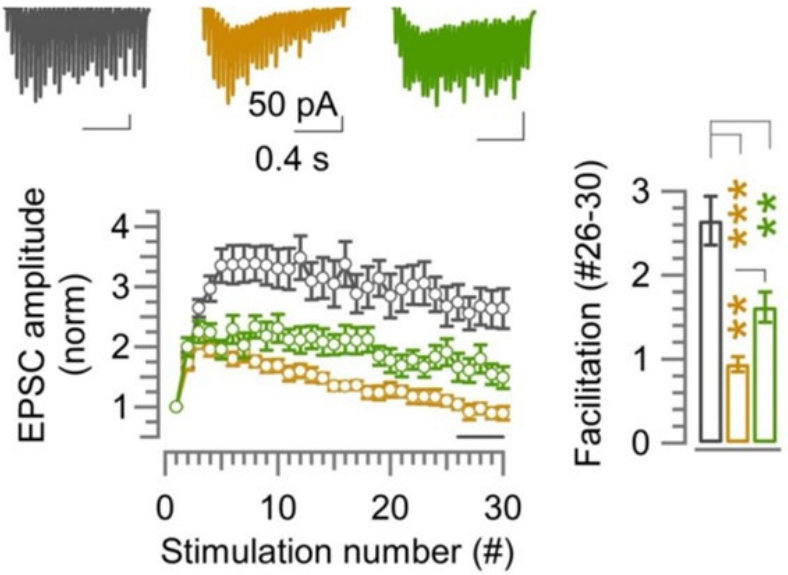


Fig 2 EPSCs at hippocampal CA1 neurons evoked by Schaffer collateral stimulation at 25 Hz.



Block of scaffold protein activity using ML-141 or Latrunculin-B likewise enhanced synaptic depression at the calyx at both 100 Hz and 10 Hz (Fig 3) but had no effect at the hippocampal synapse (Fig 4).

Fig 3 EPSCs at the calyx of Held evoked by a train of fiber stimulations.

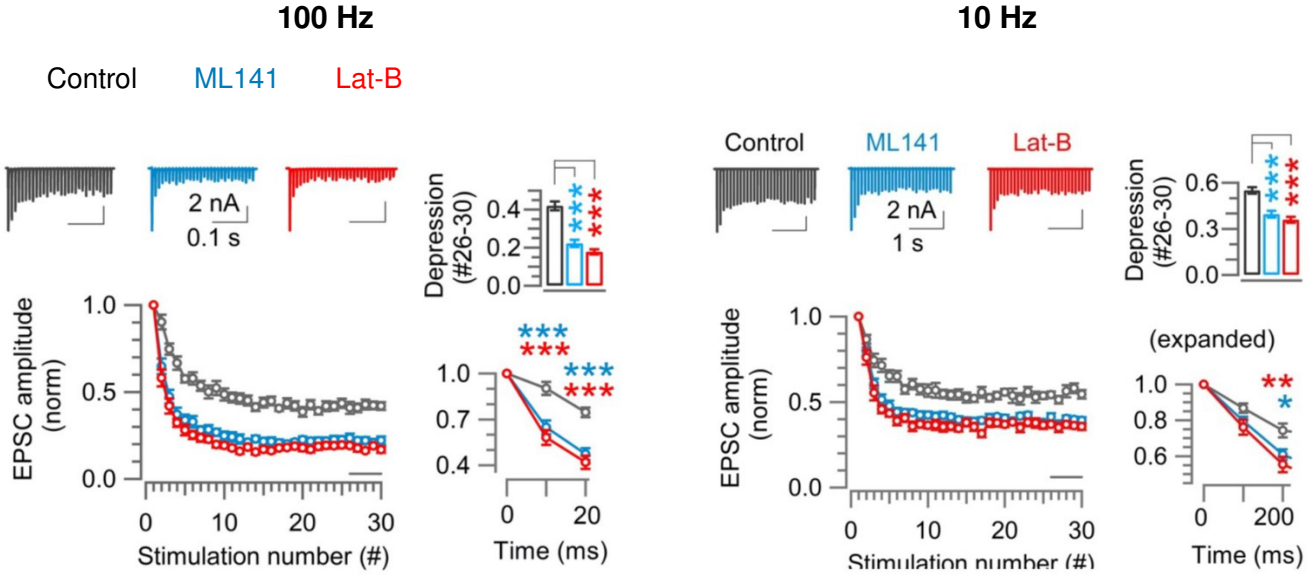
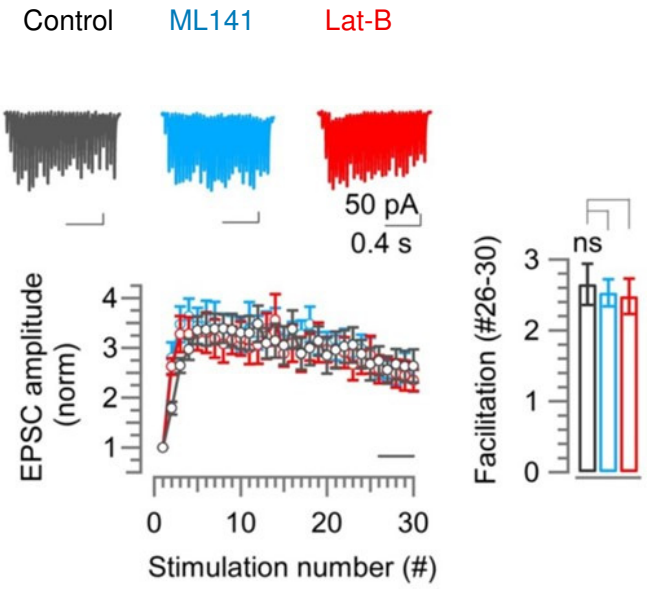
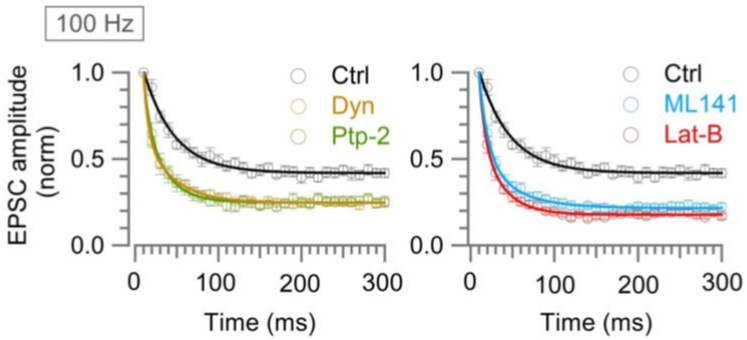


Fig 4 EPSCs at hippocampal CA1 neurons evoked at 25 Hz.



At the fast calyx synapse, block of endocytosis or scaffold protein activity significantly enhanced synaptic depression as early as 10 ms after the onset of stimulation at 100 Hz (Fig 5).

Fig 5 EPSCs at the calyx of Held evoked by a train of fiber stimulations.



100 Hz	τ_{fast}	τ_{slow}	τ_{mean}	A_f/A_s
Ctrl	-	-	37 ms	-
Dyn Ptp-2	5 ms 6 ms	35 ms 24 ms	11 ms 13 ms	0.90 0.44
ML141 Lat-B	10 ms 7 ms	40 ms 26 ms	18 ms 13 ms	3.1 2.2

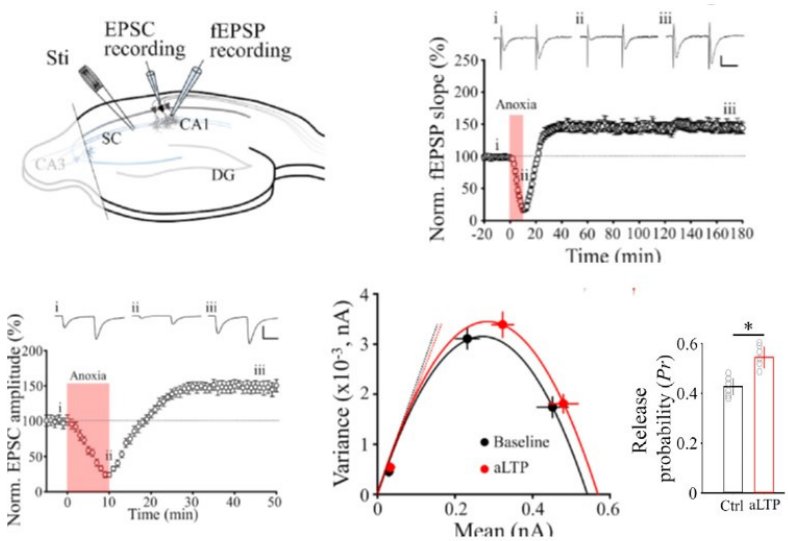
We conclude that the activity-dependent release-site clearance by fast endocytosis can be a universal phenomenon supporting vesicle replenishment at both fast and slow synapses, whereas the presynaptic scaffold mechanism likely plays a specialized role in activity-independent vesicle replenishment predominantly at fast synapses.

3.2 Anoxia-induced hippocampal LTP is regeneratively produced by glutamate and nitric oxide from the neuro-glial-endothelial axis

Han-Ying Wang, Hiroshi Takagi, Patrick N Stoney, Anai Echeverria, Bernd Kuhn, Kuei-Sen Hsu, and Tomoyuki Takahashi (*iScience* 2024).

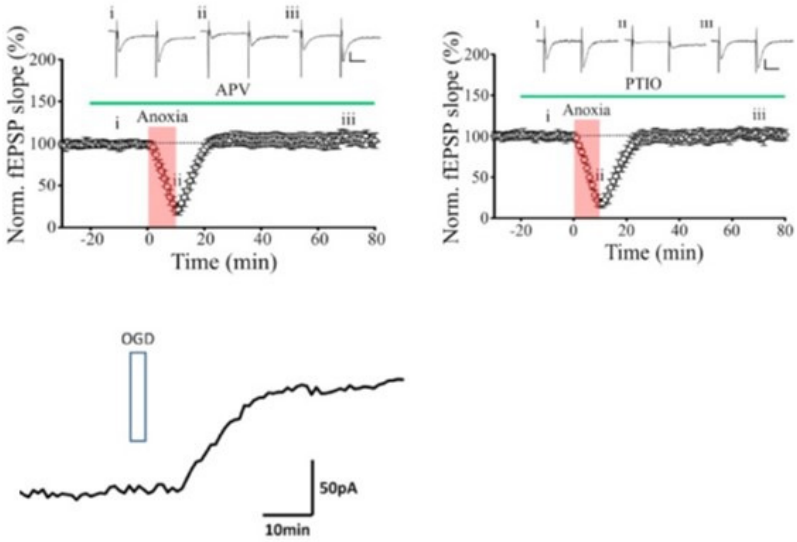
Following transient brain ischemia caused by a stroke or vascular injury, cell death occurs selectively in hippocampal pyramidal neurons in the CA1 area (Kirino et al, 1986; Zola-Morgan et al, 1986) and this is associated with anterograde amnesia reflecting working memory impairment (Zola-Morgan et al, 1986). Anoxia enhances glutamate leakage and release and elevated extracellular glutamate enhances Ca^{2+} influx through NMDA receptors, thereby activating Ca^{2+}-dependent protease and phospholipase to cause cell death (Choi, 1988). Post-anoxic enhancement of glutamate release from presynaptic terminals is modeled in rodent hippocampal slices, where transient deprivation of oxygen and/or glucose (OGD) induces long-term potentiation (LTP) of glutamatergic excitatory synaptic transmission at CA1 synapses associated with enhanced release probability as revealed by the mean variance analysis (Fig 1).

Fig 1 aLTP at the SC-CA1 pyramidal cell synapse



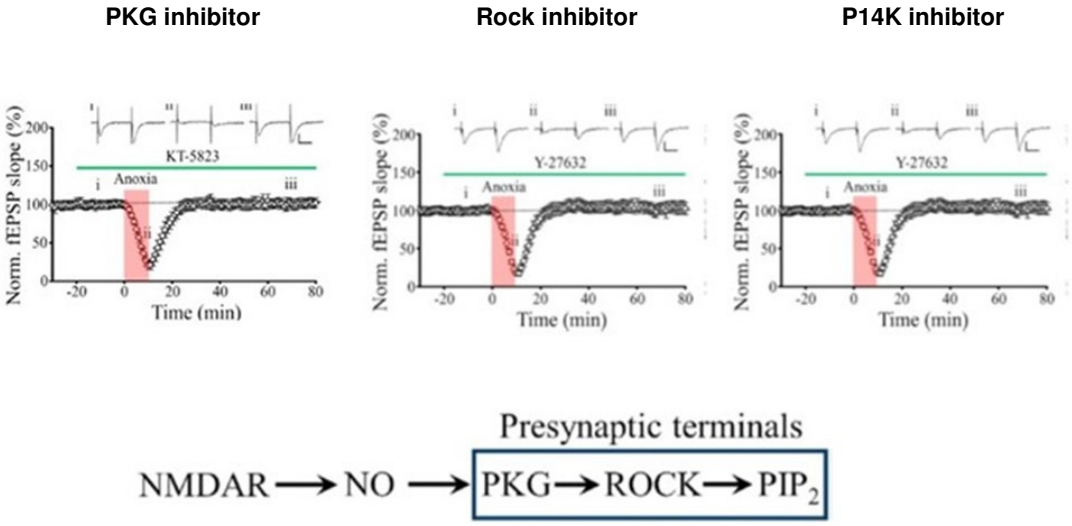
aLTP induction can be blocked by the NMDA receptor antagonist 2-amino-5- phosphonovaleric acid (APV) or a nitric oxide scavenger PTIO, suggesting the involvement of NMDA receptors and •NO in aLTP induction, and indeed •NO was released in responses to transient anoxia as shown ampero-metrically (Fig 2).

Fig 2 NMDA receptor and NO dependent nature of aLTP



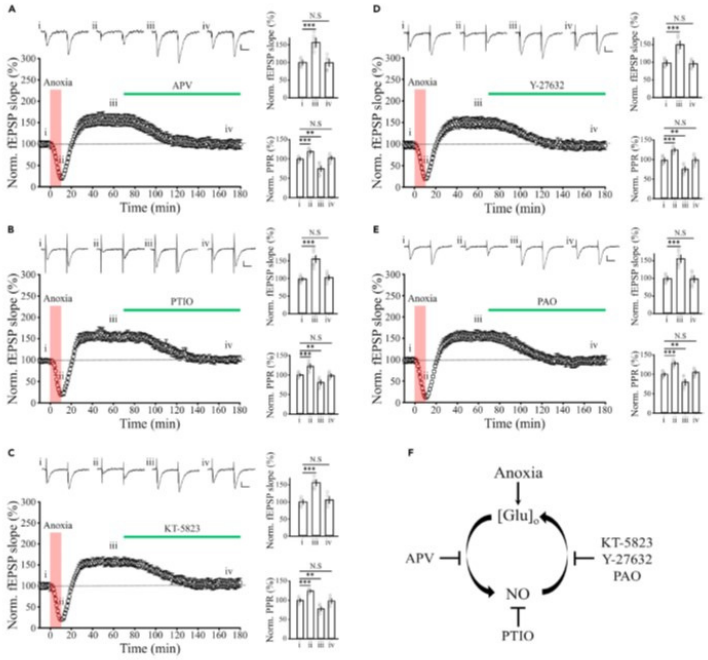
Blocking activity of •NO-downstream cascade molecules such as PKG, Rock, or PIP2 blocked aLTP induction, suggesting that transmitter release probability is enhanced by •NO activating the downstream cascade comprising PKG, Rock and PIP2 (Fig 3)

Fig 3 PKG/Rock/PI4K dependent induction of aLTP



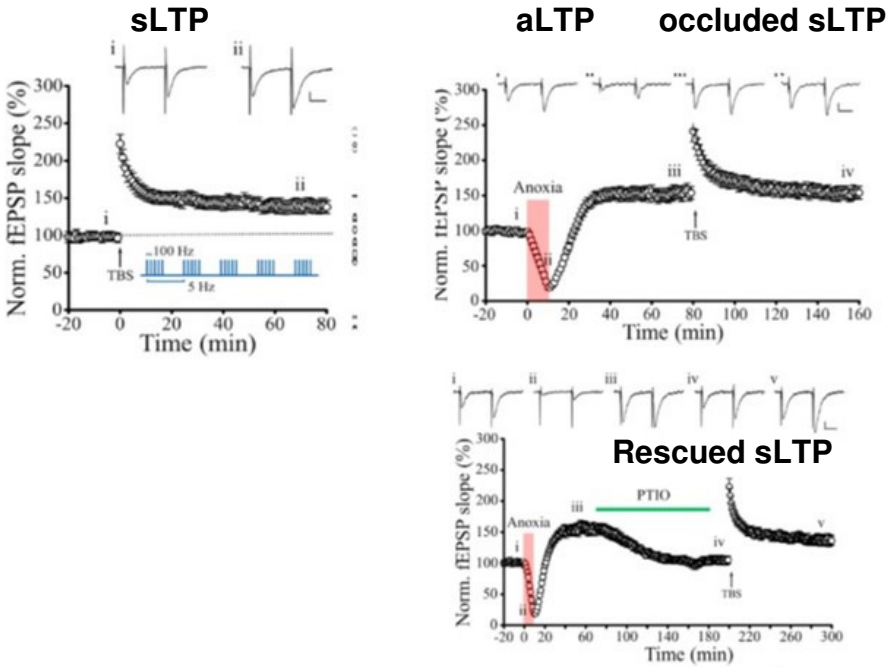
All these aLTP induction blockers also cancelled aLTP after its expression (Fig 4) suggesting that aLTP expression is maintained by repetitive inductions. Namely, glutamate released from presynaptic terminals after transient anoxia activates •NO production through activating NMDA receptors. •NO thus produced propagate into presynaptic terminals, thereby activating the •NO-downstream cascade for boosting glutamate release. Thus, a regenerative positive feedback loop is activated to maintain aLTP for many hours.

Fig 4 Regenerative production of aLTP by a positive feedback loop comprising extracellular glutamate and NO



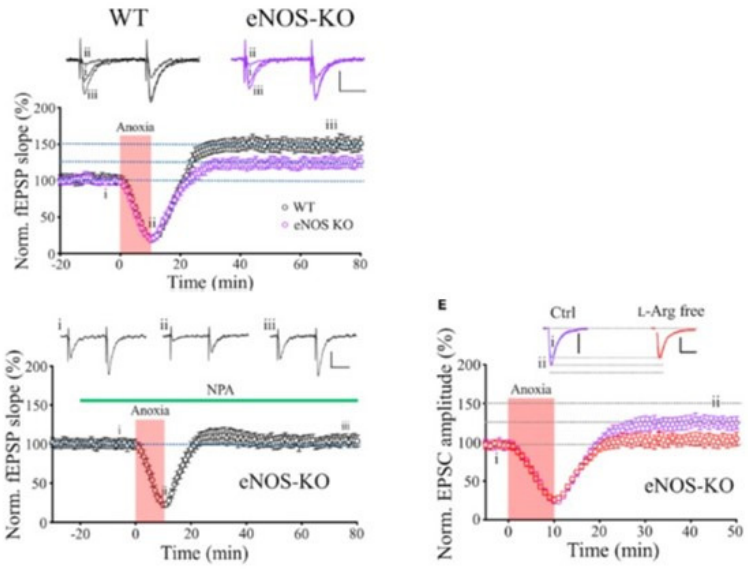
Once aLTP is expressed it occludes the stimulation-induced LTP (sLTP), which is known to underlie learning and memory (Fig 5), presumably due to occlusion at the step of NMDA receptor activation, which is also indispensable for sLTP induction. Blocking aLTP expression by PTIO rescued sLTP.

Fig 5 Occlusion of sLTP by aLTP



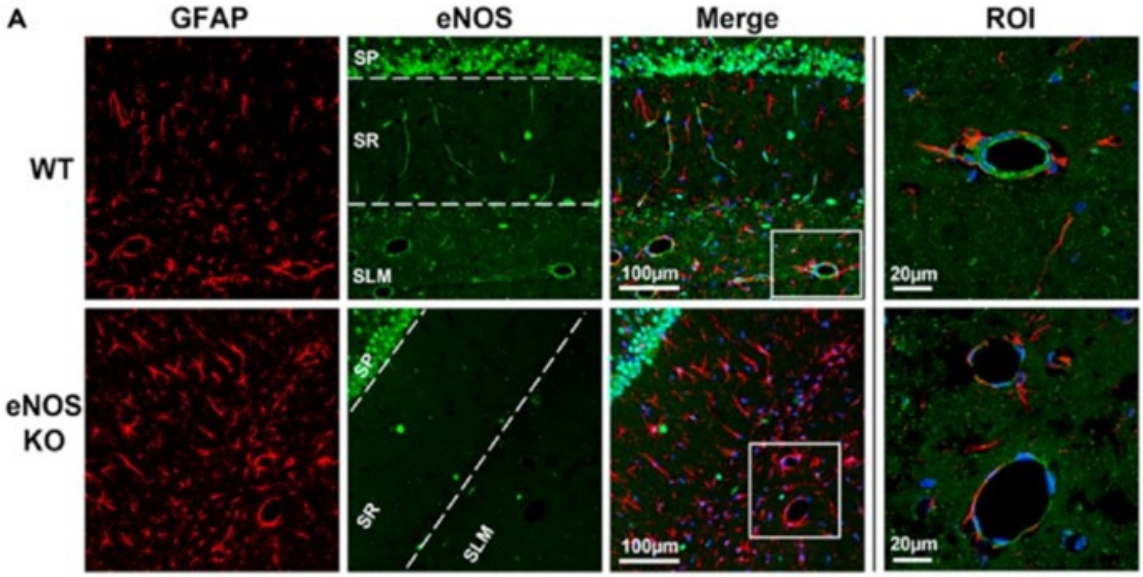
NO can be produced by neuronal •NO synthase (nNOS) or endothelial•NO synthase (eNOS). In eNOS KO mice, aLTP was half in size of that in WT mice (Fig 6). aLTP induced in eNOS KO mice was blocked by the nNOS-specific inhibitor NPA or by blocking nNOS using intraneuronal L-Arg washout, suggesting that both nNOS and eNOS contribute to aLTP production.

Fig 6 NO produced by both neuronal and endothelial NO synthase induces aLTP



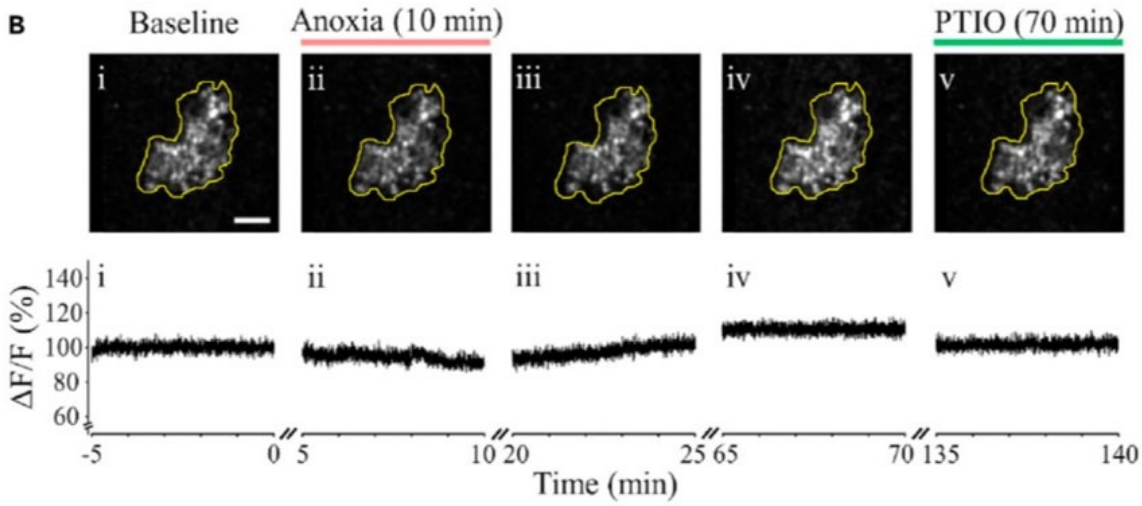
Immunocytochemical analysis of hippocampal tissue using eNOS antibody in WT and eNOS KO mice indicated that eNOS is predominantly expressed in blood vessel endothelia (Fig 7).

Fig 7 eNOS is absent in astrocyte, but present in vascular endothelia



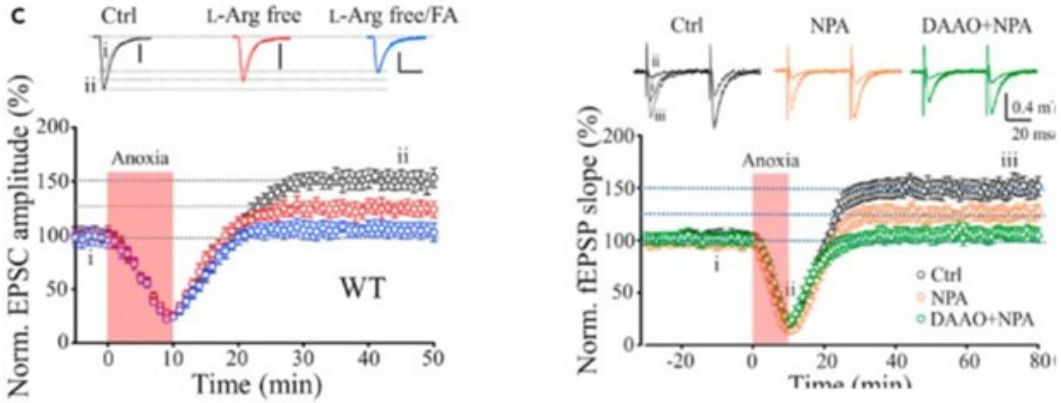
Astrocytes extend its processes to synaptic cleft on one hand and to blood vessels on the other hand, thereby forming the blood brain barrier. Two-photon Ca^{2+} imaging quantification in astrocytes indicated that astrocytic Ca^{2+} transients underdo long-term elevation mirroring neuronal aLTP (Fig 8).

Fig 8 Astrocytic calcium transient induced during aLTP



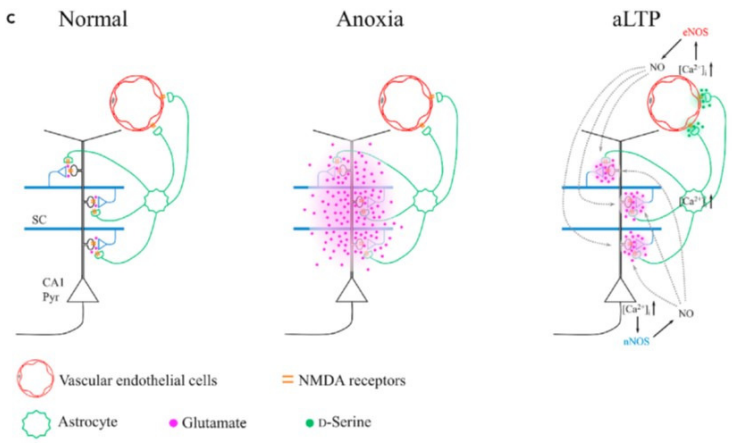
Blocking astrocyte metabolism using FA or depletion of the NMDA receptor ligand D-serine using DAAO abolished the eNOS-dependent component of aLTP, suggesting that intra-astrocytic Ca^{2+} elevation stimulates D-serine release from endfeet to endothelia, thereby releasing •NO synthesized by Ca^{2+}/calmodulin-activated eNOS (Fig 9).

Fig 9 D-serine is involved in aLTP induced by eNOS



Thus, the neuro-glial-endothelial axis is involved in long-term enhancement of glutamate release after transient anoxia (Fig 10).

Fig 10



Astrocytes contact vascular endothelia with their endfeet and astrocytic Ca^{2+} waves are propagated to their endfeet. In functional hyperemia, neurovascular coupling drives vasodilation in response to synaptic activity. This coupling is mediated by astrocytic Ca^{2+} elevation triggering release of glutamate and D-serine from astrocytic endfeet to activate endothelial NMDA receptors, thereby stimulating eNOS to synthesize •NO. Our results suggest that the same neuro-glial-endothelial coupling underlies eNOS-dependent aLTP. Since the diffusion coefficient of •NO is 3300μm^2/s (Steinert et al, 2008), •NO propagates at 115 μm/s (calculated from the mean square displacement = 4Dt). This is fast enough for •NO from endothelia to reach CA1 synapses for aLTP induction, which takes tens of minutes. Thus, the neuro-glial-endothelial coupling likely mediates dual pathophysiological functions; vasodilation and aLTP production. The former provides physiological support of brain metabolic demand, whereas the latter operates for impairing synaptic plasticity after ischemic insult.

3.3 The microtubule-dynamin binding inhibitor peptide PHDP5 rescues spatial learning and memory deficits in Alzheimer's disease model mice

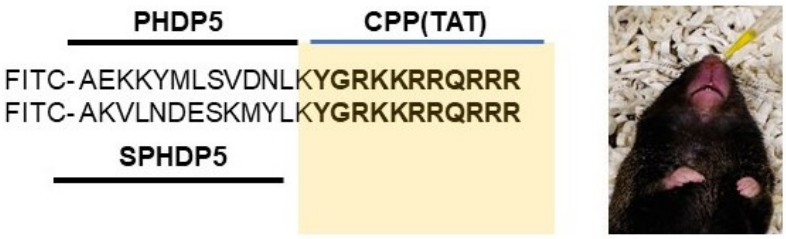
Chia-Jung Chang, Zacharie Taoufiq, Hiroshi Yamada, Takei Kohji, Takami Tomiyama, Tomohiro Umeda, Tetsuya Hori, Tomoyuki Takahashi (*Brain Research* 2024)

In neurons, tau proteins normally assemble and stabilize microtubules (MTs). Elevated soluble tau monomers accumulate and eventually precipitate into the neurofibrillary tangle (NFT), which is a hallmark of tauopathies including Alzheimer's disease (AD). The soluble tau level is correlated with AD progression and cognitive decline. Dynamin is a MT binding protein playing a key role in vesicle endocytosis. In a brain slice model, tau loaded in presynaptic terminals assembles MTs, thereby impairing vesicle endocytosis via depletion of cytosolic dynamin. As we reported previously (Hori et al, 2022, eLife), the peptide PHDP5, derived from the pleckstrin homology domain of dynamin 1, inhibits dynamin-MT interaction and rescues endocytosis and synaptic transmission impaired by tau when co-loaded in presynaptic terminals. We tested

whether in vivo administration of PHDP5 could rescue their learning/memory deficits observed in Alzheimer's disease (AD) model mice.

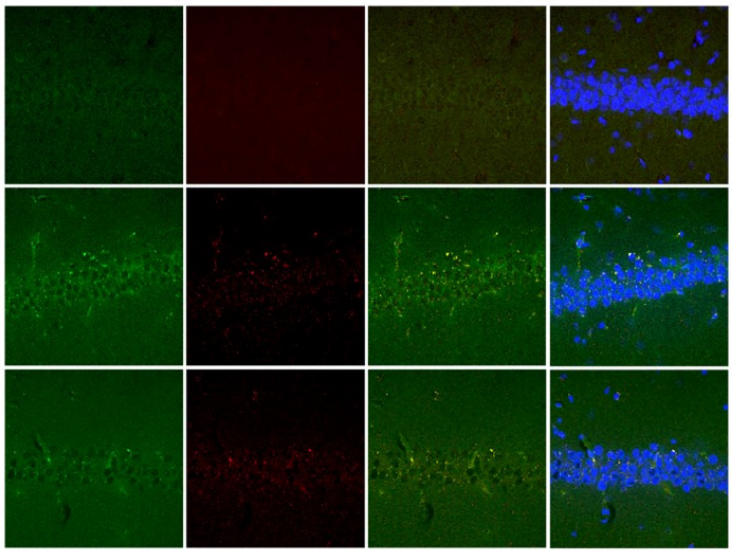
PHDP5, together with its scramble (SPHDP5),was modified by incorporating a cell-penetrating peptide (CPP) and a FITC fluorescent marker and delivered intranasally once/day for 20 days within a month to 6 month old Tau609 transgenic (Tg) and 3xTg-AD mice (Fig 1).

Fig 1 PHDP5 conjugated with FITC and CPP and its scramble infused intranasally to mice



FITC-positive puncta were observed in the hippocampus of mice infused with PHDP5 or SPHDP5 peptide, but not in saline-infused controls (Fig 2).

Fig 2 PHDPH identified by FITC in hippocampal area after intranasal delivery



In the Morris water maze (MWM) test for spatial learning/memory, AD model mice treated with FITC-PHDP5-CPP showed prominent improvements in learning and memory, performing close to the level of saline-infused WT mice control. In contrast, mice treated with a scrambled construct (FITC-SPHDP5-CPP) showed no significant improvement.

Fig 3 MWM test for learning and memory in two types of AD model mice both rescued by PHDP5 administration

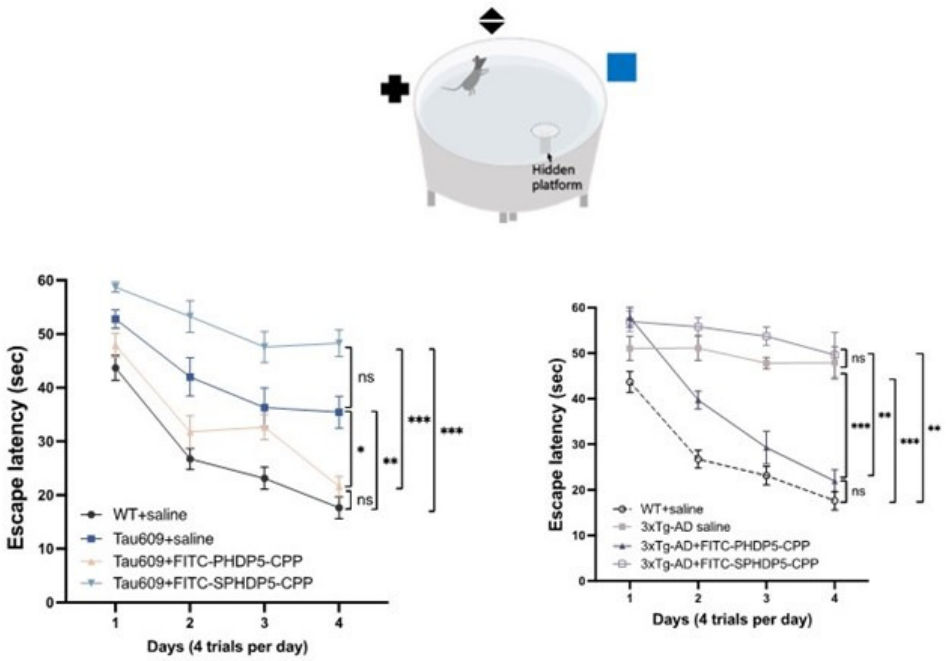
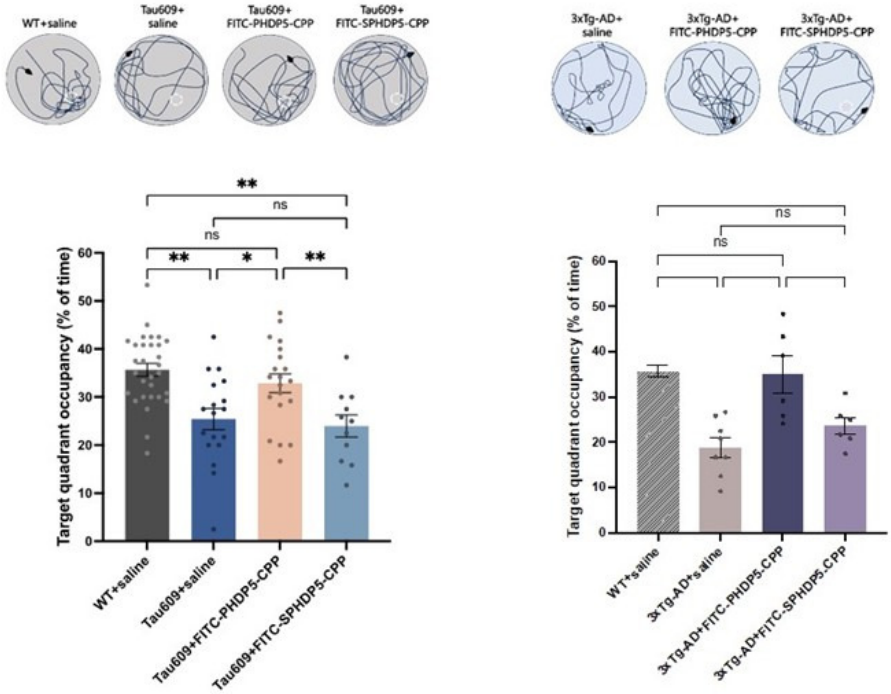


Fig 4 The probe tests for memory retention in the absence of platform



We conclude that PHDP5 can be a promising candidate for human AD therapy.

4. Publications

4.1 Journals (OIST researchers are underlined)

1. Mahapatra S and Takahashi T (2023) *Physiological roles of endocytosis and presynaptic scaffold in vesicle replenishment at fast and slow central synapses.* ***eLife*** RP90497 https://doi.org/10.7554/eLife.90497.2

2. Wang HY, Takagi H, Stoney PN, Echeverria A, Kuhn B, Hsu KS, and Takahashi T (2024) *Anoxia-induced hippocampal LTP is regeneratively produced by glutamate and nitric oxide from the neuro-glial-endothelial axis.* ***iScience*** 27,109515. DOI: 10.1016/j.isci.2024.109515

3. Chang CJ, Taoufiq Z, Yamada H, Takei K, Tomiyama T, Umeda T, Hori T, Takahashi T (2024) The *microtubule-dynamin binding inhibitor peptide PHDP5 rescues spatial learning and memory deficits in Alzheimer's disease model mice.* ***Brain Research*** 1838, 148987. DOI: 10.1016/j.brainres.2024.148987

4.2 Books and other one-time publications

Nothing to report

4.3 Oral and Poster Presentations

Oral Presentation

1. Takahashi T. *"Cellular molecular synaptic approach to neuronal disease."* In the 64th Annual Meeting of the Japanese Society of Neuropathology and the 66th Annual meeting of the Japanese Society for Neurochemistry. July 6th, 2023

2. Takahashi T. *"Calcium and CaMKII dependent mobilization of reserve and recycling synaptic vesicles." in* 細胞システム理解のためのシグナル応答原理解明の最前線; Frontiers of elucidating signal response principles towards cell system understanding in National Institute for Physiological Sciences (NIPS), Japan. September 14th-15th, 2023

3. Taoufiq Z. *"Smart & Deep exploration of subcellular proteomes."* in the JST PRESTO-OIST joint meeting in Okinawa. January 22nd, 2024

Poster presentation

1. Chang CJ, Taoufiq Z, Hori T, Takahashi T *"Evaluation of a microtubule-dynamin binding inhibitor peptide (PHDP5) as a potential tool for rescuing cognitive impairment in transgenic tauopathy mice".* AD/PD ™ 2024 advance in science and therapy in Lisbon Congress Center, Lisbon, Portugal. March 5-9, 2024

5. Intellectual Property Rights and Other Specific Achievements

Nothing to report

6. Meetings and Events

Neuroscience Symposium "The past and the future of synaptic physiology" hosted by Takahashi Unit

Date: February 13th-14th, 2024

Venue: OIST Seaside House

7. Other

Nothing to report