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Inhibition of lysine methyltransferase G9a/GLP reinstates long-term synaptic plasticity and synaptic tagging/capture by facilitating protein synthesis in the hippocampal CA1 area of APP/PS1 mouse model of Alzheimer's disease

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Histone H3 lysine 9 di-methylation (H3K9me2) is an epigenetic repressive histone modification that was found at aberrant states in Alzheimer's disease (AD) patient samples [1]. The addition of H3K9me2/3 is mainly catalyzed by lysine methyltransferase G9a, which functions as a heteromeric complex with G9a-like protein (GLP). G9a/GLP has other non-histone substrates, including itself. G9a/GLP is implicated in regulating synaptic plasticity, learning and memory [2, 3]. We have previously shown that G9a/GLP inhibition ameliorates exogenous A β oligomer-induced synaptic plasticity deficits in rat hippocampal slices [3]. In this study, we further tested the effects of G9a/GLP inhibition on synaptic plasticity.

We first tested whether G9a/GLP inhibition could rescue long-term potentiation (LTP) deficits in CA1 area of hippocampal slices of APP/PS1 mice (for materials and methods, see [supplemental file](#)). Field excitatory postsynaptic potential (fEPSP) was recorded from the stratum radiatum of CA1 and two stimulating electrodes were used to stimulate two independent Schaffer Collateral inputs (Fig. 1a). We found that a strong tetanization protocol (STET) that typically induces an input-specific late-LTP

only induced a transient potentiation (Fig. 1b). When APP/PS1 hippocampal slices were perfused with G9a/GLP inhibitor BIX-01294 (BIX, 500 nM) or UNC-0642 (UNC, 150 nM) during LTP induction by STET, a long-lasting, input-specific potentiation was observed (Fig. 1b). However, when translation inhibitor emetine (20 μ M) was co-applied with BIX or UNC, STET only led to a transient potentiation (Fig. 1c). In all recordings, the control untetanized input S2 showed no significant changes in synaptic transmission. These results suggest that G9a/GLP blockade during LTP induction could rescue input-specific synaptic potentiation in APP/PS1 hippocampal slices through upregulating protein synthesis.

Next, we examined whether G9a/GLP inhibition can restore long-term associative plasticity in hippocampal CA1 of APP/PS1 mice. The synaptic tagging and capture phenomenon is a pathway-specific form of associative plasticity in which a transient, translation-independent LTP (early-LTP) induced by weak stimulation (WTET) is stabilized into a long-lasting LTP (late-LTP) by a strong stimulation (STET) in another independent pathway within a specific time frame [3–5]. In APP/PS1 hippocampal slices, only a transient potentiation was observed after a STET and a WTET were applied 60 min apart to independent synaptic pathways S1 and S2 respectively (Fig. 1d).

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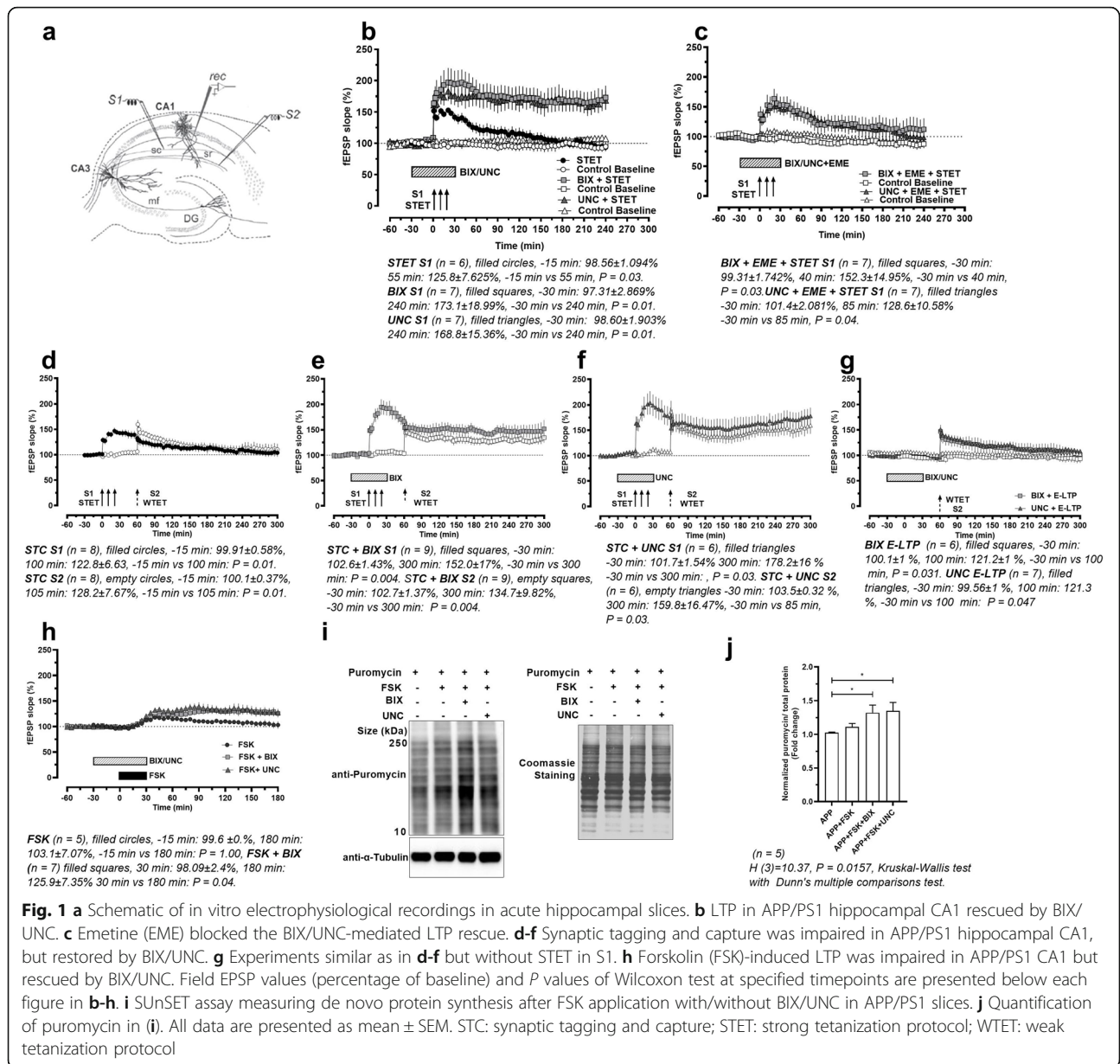


Fig. 1 **a** Schematic of in vitro electrophysiological recordings in acute hippocampal slices. **b** LTP in APP/PS1 hippocampal CA1, but restored by BIX/UNC. **c** Emetine (EME) blocked the BIX/UNC-mediated LTP rescue. **d-f** Synaptic tagging and capture was impaired in APP/PS1 hippocampal CA1, but restored by BIX/UNC. **g** Experiments similar as in **d-f** but without STET in S1. **h** Forskolin (FSK)-induced LTP was impaired in APP/PS1 CA1 but rescued by BIX/UNC. Field EPSP values (percentage of baseline) and *P* values of Wilcoxon test at specified timepoints are presented below each figure in **b-h**. **i** SunSET assay measuring de novo protein synthesis after FSK application with/without BIX/UNC in APP/PS1 slices. **j** Quantification of puromycin in **i**. All data are presented as mean ± SEM. STC: synaptic tagging and capture; STET: strong tetanization protocol; WTET: weak tetanization protocol

Then we perfused APP/PS1 hippocampal slices with G9a/GLP inhibitors (Fig. 1e-f) during STET in synaptic input S1. WTET was subsequently delivered to input S2 30 min after drug application. In both inputs S1 and S2, input-specific potentiation was rescued by G9a/GLP inhibition.

To test whether prior application of G9a/GLP inhibitors alone would transform WTET-induced early-LTP into late-LTP, experiments similar to those in Fig. 1e-f were repeated without late-LTP induction in input S1. After a 30-min washout, only a transient potentiation was observed in input S2 after WTET (Fig. 1g), in line with earlier reports that a WTET only induced a transient early-LTP.

These experiments suggest that acute G9a/GLP inhibition alone cannot induce cell-wide priming and synthesis of plasticity-related products (PRPs) in the AD-like condition. This contradicts prior studies reporting upregulation of synaptic genes and synaptic transmission in A β -impaired cortical neurons and familial AD (FAD) mice in vivo [1]. This discrepancy may be due to the duration of G9a/GLP inhibitor treatment—acute (1 h) here versus multiple days in other studies, as G9a activity can respond rapidly to external stimuli [6]. Nonetheless, it is also possible that the lack of priming by G9a/GLP inhibition [3] reflects a loss of metaplasticity in APP/PS1 hippocampus [4].

Since G9a/GLP inhibition supported late-LTP maintenance through facilitating protein synthesis in response to plasticity induction, we tested whether G9a/GLP inhibition could also facilitate chemically induced LTP by using the chemical forskolin, an activator of adenylyl cyclase, which is involved in late-phase LTP and AD pathophysiology [7].

Bath application of forskolin (50 μ M, 30 min) is typically sufficient to induce potentiation in wild-type hippocampal slices [8]. In contrast, in APP/PS1 slices, forskolin application led to an insignificant increase in fEPSP. When BIX or UNC was co-applied with forskolin, fEPSP remained stably elevated (Fig. 1h). These results suggest that G9a/GLP inhibition could also rescue slow-onset potentiation mediated by adenylyl cyclase activation.

Furthermore, we measured de novo protein synthesis after forskolin application using the SUnSET (surface sensing of translation) assay. Co-application of BIX or UNC with forskolin significantly increased de novo protein synthesis in hippocampal area CA1 (Fig. 1i-j).

Recent studies suggest that high-frequency stimulation-induced LTP and forskolin-induced LTP recruit distinct molecular pathways [9]. Here, G9a/GLP blockade ameliorated deficits in synaptic potentiation induced by both paradigms, suggesting that G9a/GLP mediates fundamental processes underlying synaptic plasticity, and that G9a/GLP is a potential therapeutic target in restoring synaptic function in AD-like conditions.

Open questions remain. Can G9a/GLP inhibition lead to the downregulation of repressive histone mark H3K9me2, thereby upregulating transcription? Are other functions of G9a/GLP, such as methylation of non-histone proteins, altered in AD? More studies are needed to clarify the precise mechanism underlying the G9a/GLP inhibition-induced upregulation of activity-dependent expression of PRPs in the APP/PS1 hippocampus.

Abbreviations

AD: Alzheimer's disease; fEPSP: Field excitatory postsynaptic potential; H3K9me2/3: Histone H3 lysine 9 di-/tri-methylation; LTP: Long-term potentiation; PRP: Plasticity-related proteins

Supplementary Information

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Additional file 1. Materials and Methods.

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Authors' contributions

J.L.T.H., K.K.L.P., M.S., and S.S. designed research; J.L.T.H., K.K.L.P., M.S., S.R.X.A. performed research; J.L.T.H., K.K.L.P., M.S. and S.S. analyzed data; and K.K.L.P., S.R.X.A. and S.S. wrote the paper. J.L.T.H. and K.K.L.P. contributed equally to this work and share first authorship. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was performed in accordance with the ethical review and received approval from the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore (IACUC protocol number: R16-0135).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Zheng Y, Liu A, Wang ZJ, Cao Q, Wang W, Lin L, et al. Inhibition of EHMT1/2 rescues synaptic and cognitive functions for Alzheimer's disease. *Brain*. 2019; 142(3):787–807.
- Gupta-Agarwal S, Franklin AV, DeRamus T, Wheelock M, Davis RL, McMahon LL, et al. G9a/GLP histone lysine dimethyltransferase complex activity in the hippocampus and the entorhinal cortex is required for gene activation and silencing during memory consolidation. *J Neurosci*. 2012;32(16):5440–53.
- Sharma M, Razali NB, Sajikumar S. Inhibition of G9a/GLP complex promotes long-term potentiation and synaptic tagging/capture in hippocampal CA1 pyramidal neurons. *Cereb Cortex*. 2017;27(6):3161–71.
- Li Q, Navakkode S, Rothkegel M, Soong TW, Sajikumar S, Korte M. Metaplasticity mechanisms restore plasticity and associativity in an animal model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2017;114(21):5527–32.
- Sharma M, Dierkes T, Sajikumar S. Epigenetic regulation by G9a/GLP complex ameliorates amyloid-beta 1-42 induced deficits in long-term plasticity and synaptic tagging/capture in hippocampal pyramidal neurons. *Aging Cell*. 2017;16(5):1062–72.
- Maze I, Covington HE, Dietz DM, LaPlant Q, Renthal W, Russo SJ, et al. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science*. 2010;327(5962):213–6.
- Vitolo OV, Sant'Angelo A, Costanzo V, Battaglia F, Arancio O, Shelanski M. Amyloid β -peptide inhibition of the PKA/CREB pathway and long-term potentiation: reversibility by drugs that enhance cAMP signaling. *Proc Natl Acad Sci U S A*. 2002;99(20):13217–21.
- Allen KD, Gourov AV, Harte C, Gao P, Lee C, Sylvain D, et al. Nucleolar integrity is required for the maintenance of long-term synaptic plasticity. *PLoS One*. 2014;9(8):e104364.
- Park AJ, Shetty MS, Baraban JM, Abel T. Selective role of the translin/trax RNase complex in hippocampal synaptic plasticity. *Mol Brain*. 2020;13(1):1–9.