Inhibiting Hippo-pathway kinases releases WWC1 to promote AMPAR-dependent synaptic plasticity and long-term memory in mice

Authors: Jens Stepan [1,2,3,4]*, Daniel E. Heinz [2,5,6], Frederik Dethloff [1,7], Svenja Wiechmann [8,9,10], Silvia Martinelli [1], Kathrin Hafner [1], Tim Ebert [2,5], Ellen Junglas [2], Alexander S. Häusl [11], Max L. Pöhlmann [11], Mira Jakovcevski [12], Julius C. Pape [1], Anthony S. Zannas [1,13,14], Thomas Bajaj [2], Anke Hermann [15], Xiao Ma [16], Hermann Pavenstädt [15], Mathias V. Schmidt [11], Alexandra Philipsen [17], Christoph W. Turck [18,19], Jan M. Deussing [12,20], Gerhard Rammes [21], Andrew C. Robinson [22,23], Antony Payton [24], Michael C. Wehr [16], Valentin Stein [25], Christopher Murgatroyd [26], Joachim Kremerskothen [15], Bernhard Kuster [8,9,10,27], Carsten T. Wotjak [5,28], Nils C. Gassen [1,2]*

Affiliations:

[1]Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry, 80804, Munich, Germany

[2]Research Group Neurohomeostasis, Department of Psychiatry and Psychotherapy, University Hospital Bonn, 53127 Bonn, Germany

[3]Department of Obstetrics and Gynecology, Paracelsus Medical University, 5020 Salzburg, Austria

[4]Department of Gynecology and Obstetrics, Technical University of Munich, 81675 Munich, Germany.

[5]Research Group Neuronal Plasticity, Max Planck Institute of Psychiatry, 80804 Munich, Germany

[6]Max Planck School of Cognition, 04103 Leipzig, Germany

[7]Metabolomics Core Facility, Max Planck Institute for Biology of Ageing, 50931 Cologne, Germany

[8]Chair of Proteomics and Bioanalytics, Technical University of Munich, 85354 Freising, Germany

[9]German Cancer Consortium (DKTK), 80336 Munich, Germany

[10]German Cancer Center (DKFZ), 69120 Heidelberg, Germany

[11]Research Group Neurobiology of Stress Resilience, Max Planck Institute of Psychiatry, 80804 Munich, Germany

[12]Department Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, 80804 Munich, Germany

[13]Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA

[14]Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA

[15]Department of Medicine D, Division of General Internal Medicine, Nephrology, and Rheumatology, University Hospital Münster, 48149 Münster, Germany

[16]Research Group Cell Signalling, Department of Psychiatry and Psychotherapy, LMU University Hospital, LMU Munich, 80336 Munich, Germany

[17]Department of Psychiatry and Psychotherapy, University Hospital Bonn, 53127 Bonn, Germany

[18]Proteomics and Biomarkers, Max Planck Institute of Psychiatry, 80804 Munich, Germany

[19]Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, 650223 Yunnan, China

[20]Research Group Molecular Neurogenetics, Max Planck Institute of Psychiatry, 80804 Munich, Germany

[21]Department of Anaesthesiology and Intensive Care Medicine, Klinikum rechts der Isar, Technical University of Munich, 81675 Munich, Germany

[22]Division of Neuroscience, Faculty of Biology, Medicine and Health, School of Biological Sciences, The University of Manchester, Salford Royal Hospital, Salford M6 8HD, UK

[23]Geoffrey Jefferson Brain Research Centre, Manchester Academic Health Science Centre (MAHSC), Salford M6 8HD, UK

[24]Division of Informatics, Imaging and Data Sciences, The University of Manchester, Manchester M13 9NT, UK

[25]Institute of Physiology II, Medical Faculty University of Bonn, 53115 Bonn, Germany

[26]Department of Life Sciences, Manchester Metropolitan University, Manchester M15 6BH, UK

[27]Bavarian Center for Biomolecular Mass Spectrometry, Technical University of Munich, 85354 Freising, Germany

[28]Central Nervous System Diseases Research, Boehringer Ingelheim Pharmaceuticals GmbH & Co. KG, 88397 Biberach an der Riß, Germany

*Corresponding authors. Emails: jens-stepan@mri.tum.de (J.S.), nils.gassen@ukbonn.de (N.C.G.).

Abstract:

The localization, number, and function of postsynaptic AMPA-type glutamate receptors (AMPARs) are crucial for synaptic plasticity, a cellular correlate for learning and memory. The Hippo pathway member WWC1 is an important component of AMPAR hetero-protein complexes. However, genetic analysis suggests that the availability of WWC1 is constrained by its interaction with the Hippo kinases LATS1 and LATS2 (LATS1/2). Here, we explored the biochemical regulation of this interaction and found that it is pharmacologically targetable in vivo. In primary hippocampal neurons, phosphorylation of LATS1/2 by the upstream Hippo kinases MST1 and MST2 (MST1/2) enhanced the interaction between WWC1 and LATS1/2, thereby sequestering WWC1. Pharmacologically inhibiting MST1/2 in male mice and in human brain organoids

promoted the dissociation of WWC1 from LATS1/2, leading to an increased abundance of WWC1 in AMPAR complexes. MST1/2 inhibition enhanced synaptic transmission in mouse hippocampal brain slices and improved cognition in healthy male mice and in male mouse models of Alzheimer's disease and ageing. Thus, compounds that disrupt the interaction between WWC1 and LATS1/2 might be explored for development as cognitive enhancers.

INTRODUCTION

Learning and memory depend on long-lasting changes in the strength of synaptic transmission, which are mediated by various molecular mechanisms, including the number, spatial organization, and function of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and their associated proteins in the postsynaptic membrane (*1, 2*). Increasing evidence links AMPARs to diseases such as Alzheimer's disease (AD) and epilepsy, making it a potential target for pharmacological intervention (*3, 4*). However, clinical trials have largely failed to consistently demonstrate clinical improvement using agonists or positive allosteric modulators of AMPARs (*5, 6*).

Prior studies have established the involvement of the WW and C2 domain-containing 1 protein (WWC1, also referred to as KIBRA) in the regulation of AMPAR and its impact on learning and memory in mice (*4, 7-13*). Papassotiropoulos *et al.'s* genetic association study played a pioneering role in establishing the significance of WWC1 in human memory, highlighting its association with memory performance through various polymorphisms in numerous human studies (*14-17*). WWC1 is well-known for its role in the evolutionarily conserved serine/threonine kinase Hippo signaling cascade (*18-20*). In concert with the core kinases mammalian sterile 20-like protein kinases 1 and 2 (MST1/2, also known as STK3/4) and the large tumor suppressor kinases 1 and 2 (LATS1/2) and the key transcription factors, Yes-associated protein (YAP1) and WW domain-containing transcription regulator protein 1 (WWTR1), WWC1 plays a central role in organ growth and regeneration (*18-21*). Numerous studies demonstrate that WWC1 is also a key component of AMPAR complexes that are enriched at postsynaptic sites in brain regions relevant to learning and memory (*4, 7-11, 14, 22-25*). WWC1 has various protein and lipid interaction domains (*11, 14, 24, 25*) that allow it to scaffold multiple binding partners including potent AMPAR regulators, such as C-kinase 1 (PICK1) (*7*), Dendrin (*25*), Protein kinase C, zeta (PKMζ) (*12, 26*), and various structural proteins of the cytoskeleton (*4, 9, 13, 22, 24, 27*).

We previously demonstrated that disrupting the interaction between Hippo pathway members WWC1 and LATS1/2 genetically, promotes the formation of WWC1-containing AMPAR scaffolds, which improves long-term memory in mice (*9*). Here we demonstrate that the interaction between WWC1 and LATS1/2 is amendable to pharmacological interventions. Inhibition of the upstream kinase MST1/2 with a small molecule drug decreased the phosphorylation of LATS1/2 (pLATS1/2), which resulted in reduced binding between WWC1 and LATS1/2. This inhibition also led to an increase in the number of postsynaptic AMPARs in both, mice and 3D human brain organoids. The same treatment improved learning and memory in male adult and aged mice, as well as in a male mouse model of Alzheimer's disease (AD). Furthermore, higher amounts of pLATS1/2 were observed in aged and AD mice, along with post-mortem brain tissue from AD patients. These findings provide mechanistic insights into the

regulation of WWC1 at synapses. Pharmacological reduction of MST1/2 activity and/or WWC1-LATS1/2 binding have the potential to reverse aging and disease-related loss of AMPAR signaling, reshape information flow through damaged neuronal networks and improve cognition.

RESULTS

Pharmacological Reduction of LATS1/2 Phosphorylation Primes AMPAR Regulation

We previously demonstrated that WWC1-LATS1/2 interaction is critical for AMPAR regulation and hippocampal learning (*9*). Antagonizing the WWC1-LATS1/2 interaction by hippocampal overexpression of WWC1 that harbors a double point mutation (Pro37/84Ala) in two WW domains crucial for LATS1/2 binding results in enhanced interaction with AMPAR subunit glutamate receptor 1 (GLUA1), key AMPAR binding partners and improved long-term memory in mice (*7, 9, 12, 28*). Here, we investigated WWC1-LATS1/2 binding in more detail. LATS1/2 and WWC1 interact at the level of the PPxY (Pro Pro x Tyr) motifs of LATS1/2 and the WW domain of WWC1 (fig. S1A) (*9*), which is essential for the regulation of the Hippo signaling cascade (*19, 21, 29*). Previous data suggest that phosphorylation events determine WWC1-LATS1/2 interaction (*29*). Using a non-phosphorylatable mutant (Thr1042Ala) or a phosphomimetic mutant (Thr1042Glu) in transfected hippocampal neurons, we demonstrated that phosphorylation of LATS1/2 at Thr^{1042} increases LATS1/2-WWC1 interaction (Fig. 1, A and B and fig. S1A). To interfere with LATS1/2 phosphorylation in vivo, we blocked the upstream kinases MST1 and MST2 (MST1/2) (*30*) with the small molecule inhibitor XMU-MP1 (hereafter, MSTi) (*31-33*). We confirmed that administration of MSTi to mice (3 mg kg^{-1}, i.p., used in all in vivo experiments) led to reduced phosphorylation of LATS1 (at Thr1079), LATS2 (at Thr1042) and the MST1/2 substrate Mps one binder1 (MOB1) (at Thr35) (*34*) in the hippocampus (Fig.1, C and D). A proteome-wide analysis of kinase inhibition in SH-SY5Y neuroblastoma cell line using the kinobead assay identified MST1/2 kinases as two major targets of MSTi (Fig. 1E, fig. S1B and Data file 1) (*35*). Previously, a link between the phosphorylation of p53 and MST1 has been established (*36*). However, we did not observe any effects of MST1/2 inhibition on phospho-p53 (fig. S1, C and D). Co-immunoprecipitation (Co-IP) experiments confirmed that the pharmacological reduction of LATS2 phosphorylation by MST1/2 inhibition strongly reduced the binding of WWC1 to LATS2 (Fig. 1, F and G and fig. S1, E and F), whereas interactions with the known AMPAR-relevant WWC1-binding partners PKMζ, Glutamate Receptor Interacting Protein 1 (GRIP1), PICK1 and GLUA1 were enhanced (Fig. 1, F and G). In contrast, we failed to observe off-target effects on end points of the Hippo pathway in brain (hippocampus, prefrontal cortex) and peripheral (liver) tissue by qPCR screening for Ctgf and Cyr61, two known targets of YAP1 transcriptional activity (*19, 20*) (fig. S2, A to C). Therefore, MSTi can serve as a precise pharmacological tool to segregate WWC1 from LATS1/2 and to strengthen its impact on GLUA1 abundance and AMPAR regulating proteins without affecting down-stream targets of the Hippo pathway. Validation of relevant AMPAR-associated WWC1 interactors revealed common molecular consequences between the pharmacological (MSTi) and the previously published genetic approach (WWC1 Pro37/84Ala) for WWC1-LATS1/2 segregation (9), shown by similar increases of nearly all validated proteins (Fig. 1, F to H, fig. S3, and data file S2). We also investigated MSTi effects on the WWC1 interactor transmembrane AMPA receptor regulatory protein γ-8 (TARPγ-8) and Calcium/calmodulin-dependent protein kinase type II subunit alpha

(CAMKIIα), two proteins critically important for memory processes (*1*). Similar to WWC1 Pro37/84Ala neurons (*9*), we found that CAMKIIα-dependent TARPγ-8 phosphorylation is strongly enhanced in primary hippocampal neurons in the presence of MSTi compared to vehicle (Fig. 1H and fig. S4A). Next, we investigated the effects of MSTi in spatial and non-spatial memory using the Morris Water Maze (MWM) and the Novel Object Recognition (NOR) test, respectively. The MWM is a widely used spatial learning test designed for rodents, wherein the subjects utilize distal cues to navigate from designated start locations along the perimeter of an open swimming arena, aiming to locate a submerged escape platform. Spatial learning is evaluated through repeated trials, and reference memory is determined by the preference for the platform area even in its absence (*37*). The NOR is used for testing non-spatial memory in rodents. It evaluates various stages of memory (acquisition, consolidation, and recall) and exploits the mouse's innate tendency to explore novelty (*38*). Both MWM and NOR demonstrate a robust correlation with the normal functioning of the hippocampus and adjacent brain areas in the medial temporal lobe (*37, 38*). To exclude the potential impact of the injection procedure, we administered MSTi to the animals both, before and after the daily training sessions. Administration of MSTi before and after daily MWM training with an inefficient protocol modified to two learning trials per day (*9, 39*), facilitated the formation of long-term spatial memory (Fig. 2, A and B and fig. S4, B to D, G and H). Likewise, MSTi, but not vehicle pre- and post-treated mice were able to remember the novel object for at least 24 h (fig. S4, E, F, I and J).

Drug-induced facilitation of AMPAR trafficking and synaptic transmission

To test the hypothesis that WWC1 affects learning and memory through changes in AMPAR trafficking, we examined the number of postsynaptic AMPARs in hippocampal slices after treatment with MSTi, using biotinylation assays in combination with Western blots (*40*) (WBs). Treatment of brain slices with MSTi (3 μM, all in vitro experiments) but not vehicle increased the number of AMPAR subunits GLUA1 and GLUA2, but not GLUA3, at the plasma membrane of hippocampal neurons (Fig. 2, C and D). Moreover, electrophysiological measurements showed that MSTi increased the frequency, in particularly of low-amplitude events (Fig. 2, E and F and fig. S5, A to G), but not the amplitude of miniature excitatory postsynaptic currents (mEPSCs), recorded in hippocampal CA1 pyramidal cells (Fig. 2G and fig. S5, A to G). MSTi had no effect on the paired-pulse ratio of Schaffer collateral-CA1 field-excitatory post-synaptic potentials in mouse hippocampal brain slices (fEPSPs) (fig. S5H). Together, these data suggest an increased number of functional AMPARs and/or AMPARs emerging from below the detection threshold (*41-43*). With respect to evoked neuronal activity, MSTi induced a fast and robust increase in fEPSPs recorded from the CA1 region of hippocampal slices following stimulation of the Schaffer collaterals (fig. S5I). This effect was sustained for 180 min following washout of the drug after 25 min (Fig. 2, H and I) and did not obstruct the subsequent induction of long-term potentiation (LTP) (Fig. 2J), indicating that MSTi does not saturate AMPAR-dependent synaptic plasticity. At network level, voltage-sensitive dye imaging (VSDI) (*44*) revealed enhanced neuronal activity propagation through the trisynaptic hippocampal circuit (Fig. 2, K and L and fig. S5, J and K). The effects of MSTi on synaptic transmission were substantially attenuated, but still present in brain slices of *Wwc1* knock-out (*Wwc1*$^{-/-}$) mice (Fig. 2M).

At the behavioral level, genetic deletion of *Wwc1* substantially attenuated, but did not ultimately prevent spatial learning in the MWM (Fig. 2N and fig. S5, L to N) and NOR tasks (fig. S5, O and P). Comparable swimming distances were recorded in both groups during the probe

trial (fig. S5Q), indicating no differences in general motor function. Furthermore, no other obvious behavioral abnormalities, differences in coat status or body weight (fig. S5R) were observed in *Wwc1*$^{-/-}$ and *Wwc1* wild-type (*Wwc1*$^{+/+}$) mice. The preserved effects of MSTi in *Wwc1*$^{-/-}$ mice might be ascribed to compensation by the highly homologous WWC2 (*7, 21*). In fact, MSTi reduced the interaction between WWC2 and LATS1/2 in primary hippocampal neurons, whereas the binding of WWC2 to AMPAR regulators PKMζ and PICK1 was enhanced (fig. S5, S to V).

MSTi enhances learning in mouse models of cognitive decline and promotes AMPAR-WWC1 interaction in brain organoids, and mechanism is supported in human postmortem brain tissue

To further elucidate the promnesic potential of MSTi, we studied its memory-enhancing effects in male mouse models of age-related cognitive decline and Alzheimer's disease (AD). We found that MST1/2 inhibition increased neuronal activity in the hippocampus of 23-month-old wild-type mice and improved spatial and object recognition memory in both aged- and hemizygous *ArcAß* (*ArcAß*^{HEMI}) mice expressing human *APP* with the combined Swedish and Arctic mutations (*45*) (Fig. 3, A to F and fig. S6, A to E). *ArcAß*^{HEMI} mice are characterized by dramatic increases in amyloid plaques between 9 and 15 months of age, which is accompanied by cognitive impairment beginning at the age of 6 months in behavioral tests including MWM and Y-maze as well as in active avoidance behavior (*45*). The amount of pLATS1/2 seemed to be crucial for synaptic availability of WWC1, its capacity to scaffold AMPAR complexes and its promnesic potential (*9*) (Fig. 1, A to D and F to H). Accordingly, we found increased pLATS1/2 abundance in 23-month-old wild-type and *ArcAß*^{HEMI} mice (Fig. 3, G and H).

We next examined whether comparable changes in the Hippo pathway occur in human brains. Similar to the mouse models, we found increased amounts of pLATS1/2 in post-mortem brain tissue from AD patients compared to sex- and age-matched controls (*46, 47*) (Fig. 3I, fig. S6F and Data file S3). In the brains of individuals with AD, we also observed elevated binding between WWC1 and LATS1/2, coupled with a reduction in the binding of WWC1 to GLUA1 (Fig. 3, J and K and data file S3). Finally, we investigated the number of postsynaptic AMPARs in human brain organoids and found increased amounts of AMPAR subunits GLUA1 and GLUA2 at the plasma membrane after MSTi treatment (Fig. 3L). These changes in postsynaptic AMPAR subunit abundance were accompanied by decreased amounts of pLATS1/2 (fig. S6G).

DISCUSSION

Despite a growing number of studies on the roles of WWC1 in synaptic plasticity and learning in animals and humans, a targeted pharmacological approach to increase postsynaptic WWC1 is currently lacking. Here we provide compelling evidence that pharmacological blockade of MST1/2 effectively reduces phosphorylation of LATS1/2, leading to the dissociation of WWC1 from LATS1/2. This dissociation facilitates synaptic function and long-term memory in male mice (fig. S7). These results support our previous genetic experiments, confirming that the interaction between WWC1 and LATS1/2 governs the synaptic availability of WWC1 and its scaffolding capabilities (*9*). Moreover, our findings align with previous research demonstrating that overexpression of WWC1 enhances AMPAR trafficking, synaptic plasticity, and learning and memory, whereas knock-down of *Wwc1* has the opposite effect (*4, 7, 8, 25*).

Blockade of Hippo kinases MST1/2 by MSTi and the resulting segregation of WWC1 from LATS1/2 led to increased excitation of monosynaptic transmission and particularly also in greater output from the polysynaptic hippocampal network. An increased number of postsynaptic AMPARs, as revealed by an increase in GLUA1 and GLUA2 protein abundance in hippocampal membrane precipitates after MSTi treatment, can explain the fast and robust increase in mEPSCs frequency (*41-43*). Together with the unchanged presynaptic release probability and despite unaltered average mEPSC amplitudes, these data can reflect the emergence of AMPARs in postsynaptically silent synapses (*41-43*), likely by lateral diffusion from extrasynaptic to synaptic sites (*48*). Moreover, actual increments in mEPSC amplitudes facilitate the detection of previously sub-threshold events, consequently elevating the mEPSC frequency. However, the average amplitude likely remains unaffected, as a result of incorporating a larger number of small, previously undetected events into the average value (*49*). The finding that MSTi had a particularly strong effect on small amplitude events, supports such a scenario, which becomes even more likely if one considers that the unsilencing of synapses is characterized by the initial occurrence of small amplitude events (*41, 42*). The prolonged effect of MSTi on hippocampal activity may also require the supply of additional AMPARs to replenish the extrasynaptic surface pool, which can occur by recycling endosome driven delivery of AMPARs to synaptic sites (*50, 51*). The WWC1 heterocomplex includes various myosins and RAB11-FIP2 proteins that have been implicated in mobilization of recycling endosomes and AMPA receptors during synaptic plasticity (*50, 51*). The chemically induced LTP elicited by MSTi did not prevent the subsequent induction of electrical LTP, suggesting that MSTi treatment did not saturate AMPAR-dependent synaptic plasticity. Even though we cannot entirely rule out that MSTi and electrically induced LTP involve independent intracellular signaling cascades, we could show that WWC1 is intimately linked to AMPAR regulation and both processes share at least the recruitment of major postsynaptic proteins (such as PSD-95, TARPγ-8, and CAMKIIα) (*52*), and the postsynaptic membrane insertion of additional AMPARs. In conclusion, both prior research and the results of our study collectively suggest that a presynaptic effect of MSTi is unlikely.

A pathological hallmark of AD is the extracellular accumulation of beta amyloid (Aβ) - containing plaques, whose presence as diffusible oligomers is highly correlated with synaptic deterioration including aberrant AMPAR trafficking (*53*). Accordingly, ArcAβ mice exhibit strong impairment of neurotransmission and cognition early in life (*45, 54*). Similarly, aged animals show decreased number of postsynaptic AMPARs, which correlates with cognitive decline and impaired synaptic plasticity (*55, 56*). Here, pharmacological segregation of WWC1 from LATS1/2 enhanced AMPAR trafficking and enabled the formation of hippocampus-dependent long-term memories in both male aged wild-type and ArcAβ mice. The increased abundance of pLATS, along with the presumed strengthened binding between WWC1 and LATS1/2 in our animal models of cognitive decline and AD patients, contrasts with previous observations of reduced levels of WWC1 in both AD patient brain tissue and an AD animal model (*4*). By enhancing the WWC1-LATS binding, the overall quantity of WWC1 remains unaffected. This implies that the compromise in WWC1 function can arise through various mechanisms. It can result from a decrease in WWC1 abundance but also from a mechanism that reinforces the interaction between WWC1 and LATS, such as heightened MST1/2 activity. The latter hypothesis aligns with existing data illustrating cognitive decline and synaptic dysfunction arising from MST1 overexpression in the hippocampus (*57*). This is further substantiated by observations of ameliorated cognitive deficits and reduced neuronal apoptosis in another mouse model of AD (5xFAD) following genetic knock-down or inhibition of MST1 (*33*). Together these data support our results suggesting that

the dissociation of WWC1 from LATS1/2 provides a major mechanism linking Hippo signaling, AMPAR regulation and cognitive performance (fig. S7).

Treating healthy-(aged) individuals or AD patients with MST1/2 inhibitors could potentially have severe side effects. LATS kinase tightly regulates transcription factors YAP/TAZ whose pervasive activation is widespread in many human malignancies (*18*-*20*). However, MSTi did not seem to cause changes within the Hippo cascade, as assessed by qPCR of established YAP/TAZ target genes (fig. S2, A to C). Given that we cannot exclude potential adverse effects of MST1/2 inhibitors on Hippo signaling, it is essential that future agents be refined to preserve the integrity of Hippo while ensuring optimal accessibility of WWC1 for synaptic processes.

One of the major concerns associated with modulating AMPAR abundance and/or function in humans lies in the potential for inducing pro-convulsant effects (*6*). However, MSTi treatment sequesters WWC1 from LATS leaving the overall abundance of both proteins unchanged. Moreover, MSTi treatment has no effect on synaptic potentiation in LTP experiments, suggesting a submaximal activation of AMPARs. These findings may also account for the moderate effects of MSTi in both the fEPSPs and VSDI experiments. It appears that MSTi, particularly at the concentration employed in this study, activates AMPARs without lowering the threshold for epileptic potentials. This finding aligns with our observations of normal behavior in mice following MSTi treatment and the absence of uncontrolled activity in hippocampal brain slices perfused with the drug.

Our previously reported investigation (*9*) yielded important insights into the potential interactions among AMPAR subunits. Although our methodology effectively identifies proteins associated with WWC1 and AMPAR subunits, it falls short in conclusively characterizing these interactions as direct protein-protein engagements. Consequently, we can only speculate on the predominance of GLUA homo- or heterodimers. And although the MST1/2-dependent effects of MSTi were confirmed through subsequent loss-of-function experiments, and although comprehensively our data indicate that the primary mechanism of action is mediated through WWC1, it nevertheless remains possible that a fraction of the observed effects may be co-regulated by other kinases, such as TAOK2 (*58*). Addressing these questions should serve as the cornerstone for future research endeavors. In summary, the WWC1-LATS1/2 interaction emerges as a promising target for restoring AMPAR signaling in cognitive decline.

MATERIALS AND METHODS

Animals

Experiments were performed with group-housed male mice, which were maintained in IVC racks (Greenline Tecniplast; equipped with wooden enrichment tubes) under standard laboratory conditions (22C ± 3C, 50% ± 10%) humidity, food and water ad libitum, 12 hour:12 hour light-dark cycle with lights on at 06:00 hours) in the vivarium of the Max Planck Institute of Psychiatry for at least 7 days before starting with the experiments. If not stated otherwise, mice were kept on the C57BL/6N background and derived from internal breeding stocks (Max Planck Institute of Biochemistry) and tested at an age of 10-16 weeks. Aged, male C57BL/6JRj mice were purchased from Janvier at an age of 20 months and tested at an age of 22-23 months. Male *Wwc1* knock-out mice (*7, 12*) were maintained on a 129SV/C57BL6/N hybrid background. Experiments were performed on homozygous males (knock-out = $Wwc1^{-/-}$) and their age-matched littermate controls

(wild-type = *Wwc1*$^{+/+}$) at an age of 10-16 weeks. Male transgenic mice with the arctic mutation as well as the Swedish mutation, served as an AD model and were generated as previously described (*45*). Experiments were performed on hemizygous (HEM) males (*ArcAß*^{HEMI}) and their age-matched littermate controls (wild-type, *ArcAß*$^{+/+}$) at an age of 9-13 months. All animal studies agreed with the government of Upper Bavarian (AZ: ROB-55.2-2532.Vet_02-18-55) and were conducted in accordance with the recommendations of the Federation for Laboratory Animal Science Associations and according to the European Community Council Directive 2010/63/EEC.

Human samples

All brains underwent a neuropathological examination including several brain regions. These studies did not include subjects with evidence for gross and/or macroscopic brain changes, or clinical history, consistent with cerebrovascular accident or other neurological disorders. Postmortem diagnoses were determined by two clinicians on the basis of retrospective review of medical records and extensive questionnaires concerning social and medical history provided by family members. Fresh, frozen tissue was taken from the superior frontal gyrus (Brodmann area 8) of the frontal cortex from 71 brains [AD: n = 36, control (Ctrl): n = 35] of donors who were participants of a large prospective cognitive aging cohort known as the University of Manchester Longitudinal Study of Cognition in Normal Healthy Old Age Cohort (UMLCHA) (*46, 47*). Samples were used for capillary based immune blotting (ProteinSimple, Biotechne). Tissue samples were supplied by The Manchester Brain Bank, which is part of the Brains for Dementia Research programme, jointly funded by Alzheimer's Research UK and Alzheimer's Society. Groups were matched based on demographic factors (data file S3).

Drugs

The dose of XMU-MP1 (MSTi, Tocris) for all intraperitoneal (i.p.) injections was 3 mg kg^{-1}. MSTi was dissolved in DMSO (0.02%) and sterile saline. For all in vitro experiments MSTi was dissolved in dimethylsulfoxid (DSMO, 0.003%) and artificial cerebrospinal fluid (ACSF) for a final concentration of 3 µM. In all MSTi experiments, DMSO (0.003%) was added during control experiments. The pH of all solutions was adjusted to approximately 7.4.

Morris Water Maze (MWM)

The test was performed in a circular white pool with a diameter of 150 cm and 41 cm-high walls that was placed on a table so that the edge of the wall was 110 cm above the floor. The pool was filled with fresh tap water to a height of 33 cm on the first day before the first trial. The water temperature was maintained at 21 ± 1°C. The pool was localized in the middle of a cubic-like room (W309 cm x L357 cm x H283 cm), which contained prominent customized landmarks at the walls (rectangular, triangular and circular posters with different black/white patterns, fixed 170 cm above the floor), but no windows or additional prominent cues. The computer for video tracking was placed in the SW corner and a sink located in the NE corner, both not visible to the animals from the pool. The room was illuminated by indirect light with two spots facing the wall, resulting in 11.5 lux at water surface level. The escape platform of clear acryl analyzed (diameter: 10 cm) was placed in a fixed position in the northwest (NW) quadrant 1 cm beneath the water surface and 35 cm away from the wall.

Mice received intraperitoneal injection (i.p.) of MSTi 45 min before the first trial daily or after completing all trials of the day. Subsequently, mice were transported cage-wise from the holding room to the adjacent training room and placed in front of a heating lamp after completing all trials.

As indicated in the respective paragraphs of the results section and/or figure legends, each animal had to perform 2 trials per day with differing starting positions over a period of 5 to 9 days and with an inter-trial interval of 3-7 min. The starting positions were assigned in random order out of 7 positions evenly distributed along the perimeter of the pool. For each trial, mice were gently placed on the water surface facing the wall, and the experimenter took a seat at a fixed position in the room. If an animal climbed onto the platform, it remained there for further five seconds until the experimenter brought the animal back to its home cage with the help of a metal grid fixed to a stick. If an animal did not find the platform within 60 s, the experimenter guided the animal to the platform with the stick without touching the mouse and noted 61 s as escape latency.

Each trial was recorded and analyzed by ANY-MAZE (version: 7, Stoelting). We assessed the following main parameter: (i) the escape latency recorded during the first trial per day (as a measure of long-term memory formation which is not confounded by short-term memory processes at a given training day) and, (ii) escape latencies averaged over all training days. 24 h after the last training, animals performed a 60 s probe trial during which the platform was removed from the maze, and the animals were started from the quadrant opposite to the target quadrant. The video tracking software divided the pool into 4 virtual quadrants, and we assessed, (iii) the time the animals spent in each of the quadrants and (iv) the number of exact platform crossings. We also used the software option to additionally present averaged heat maps of MWM exploration that illustrate the selectivity of searching.

Novel Object Recognition (NOR)

Experiments were performed in a Y-shaped setup (arm length: 30 cm, arm width: 10 cm, wall height: 15 cm; floor covered with bedding) under low-light conditions (< 40 lux). After a 9 min habituation trial at day 0, mice were place in the base arm, with two identical objects (screw nut 6 cm x 4 cm, or bottle 7 cm x 5 cm) in the end of the upper left and upper right arms, and allowed to explore the objects for 9 min at day 1 (training). As indicated in the respective figure and/or figure legend, MSTi was injected (i.p.) 45 min before sampling or right after. One day later (day 2), mice were placed back to the setup whereby one familiar object (FO) was replaced by a novel object (NO) for 9 min. The setup was equipped with new bedding after each trial. Object type, start position and position of FO and NO were completely randomized, and all sessions were videotaped. A trained observer who was blinded to the experimental groups, object type and position scored offline the interaction time of the animals with each of the objects during the test phase. As a measure of object recognition memory, we considered both the exploration time spent with each object as well as the ratio of investigation duration (RID), which was calculated as follows: (NO-FO)/(NO+FO).

Preparation of brain slices for voltage-clamp experiments, local field potential recordings and membrane protein biotinylation studies in vitro

Male mice were anesthetized with isoflurane (Abbott) and decapitated. All following steps were done in ice-cold cutting saline saturated with carbogenated gas (95%/5% O_2/CO_2). This saline (approximately adjusted to pH 7.4) consisted of (in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 6 $MgCl_2$, and 25 D- glucose (all salts for the saline solutions were purchased from Sigma-Aldrich). After decapitation, the brain was rapidly removed from the cranial cavity and 350-µm-thick horizontal slices containing the hippocampus were cut using a vibratome (HM650V; Thermo Scientific). Afterwards, slices were incubated for 30 min in carbogenated standard recording artificial cerebrospinal fluid (ACSF) at 34°C. This saline (approximately

adjusted to pH 7.4) consisted of (in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, and 25 D-glucose. Subsequently, slices for electrophysiology were stored at room temperature for at least 30 min in carbogenated ACSF before recordings. Slices for protein analysis were processed immediately.

Membrane protein biotinylation

Hippocampal slices from male mice were recovered in ACSF (artificial cerebrospinal fluid; 125 mM NaCl, 2.5 mM KCl, 1.2 mM NaH2PO4, 1.2 mM MgCl2, 2.4 mM CaCl2, 26 mM NaHCO3, and 11 mM glucose) for 40 min while bubbling with 95%/5% O_2/CO_2. After washing (3 x with prewarmed ACSF) slices were incubated in oxygenated ACSF (95%/5% O_2/CO_2) supplemented with vehicle or MSTi (3 µM) for 45 min. Slices were washed (3 x ice cold ACSF) and biotinylated with 1 mg ml^{-1} sulfo-NHS-SS-biotin (Pierce, #21331) in ACSF on ice for 45 min. After washing (3 x ice cold ACSF) and 10 min incubation on ice slices were washed in slice quench buffer (3 x ice cold, 125 mM NaCl, 2.5 mM KCl, 1.2 mM NaH2PO4, 1.2 mM MgCl2, 2.4 mM CaCl2, 26 mM NaHCO3, and 11 mM glucose, 100 mM glycine) and incubated in slice quench buffer for two times 25 min on ice. Slices were washed again (3 x ice cold ACSF) and lysed in ice cold RIPA buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1.0 mM EDTA, 1% Triton-X-100, 0.1% SDS, 1% Na deoxycholate) supplemented with protease inhibitors (PI; Merck Millipore) for 30 min at 4°C while rotating. The centrifugation-cleared (15 min, 4°C, 18,000 x g) supernatant was used for streptavidin precipitation using magnetic streptavidin beads (Thermo, #11206D). A fraction of lysate was stored for (WB). Beads were equilibrated in RIPA (3 x at room temperature) and added to the lysates. Binding was performed by rotating the tubes (Protein LoBind Tubes 1.5 mL, Eppendorf) overnight at 4°C. Beads were washed (3 x RIPA+PI) and pellet was air dried and were eluted in 2 x SDS-PAGE reducing sample buffer (30 min at room temperature). Lysates and elutes were analyzed by (WB).

hiPSC generation and maintenance

Human induced pluripotent stem cells (hiPSC) were derived from a female donor through the BeCOME study (*59*) and reprogrammed using a plasmid-based protocol for integration-free hiPSCs from peripheral blood mononuclear cells (*60*) hiPSC were cultured at 37°C with 5% CO_2 on 6-well plates (Thermo Fisher) coated with Matrigel (1:30, Corning) in supplemented mTesR1 (Stem Cell Technologies).

Brain organoids

The protocol to generate cerebral organoids was adapted from Lancaster and Knoblich (*61*). Human iPSCs were dissociated with StemPro Accutase Cell Dissociation Reagent (Life Technologies) and embryoid bodies (EB) are started by transferring 9'000 cells into each well of a 96-well U-bottom, low-attachment plate (Corning), which counted as day 0. Ebs were maintained in hES medium (DMEM/F12-GlutaMAX supplemented with 20% knock-out Serum Replacement, 3% FBS, 1% Non-essential amino acids, 0.1 mM 2-mercaptoethanol, 4 ng/ml bFGF and 50 µM Rock inhibitor Y27632) for 6 days after which they were transferred into low attachment 24-well plates (Corning) in Neural Induction (NIM) medium (DMEM/F12 GlutaMAX-supplemented with 1:100 N2 supplement, 1% Non-essential amino acids and 5 µg/ml Heparin) After additional 6 days, each EB was embedded into a Matrigel (pure) droplet and transferred into 10 cm culture plate with Neural Differentiation medium (NDM) (DMEM/F12GlutaMAX and Neurobasal in ratio 1:1 supplemented with 1:100 N2 supplement 1:100 B27 without Vitamin A, 0.5% Non-essential amino acids, insulin 2.5 µg/ml, 1:100 Antibiotic-Antimycotic and 50 µM 2-mercaptoethanol).

After 4 days, the plates were transferred on an orbital shaker and supplemented with Vitamin A (DMEM/F12GlutaMAX and Neurobasal in ratio 1:1 supplemented with 1:100 N2 supplement 1:100 B27 with Vitamin A, 0.5% Non-essential amino acids, insulin 2.5 µg/ml, 1:100 Antibiotic-Antimycotic and 50 µM 2-mercaptoethanol).

On day 60, 8 organoids were transferred into two wells (4 organoids per well) of a 6-well plate and treated with MSTi (3 µM) or vehicle. After 45 min, the organoids were washed 3x with cold PBS and cut in half with a scalpel. Biotinylation was performed as described above.

Western blot analysis

Protein extracts were obtained by lysing cells in Pierce™ IP lysis buffer (150 mM NaCl, 1% NP-40, 1 mM EDTA, 5% glycerol, 25 mM Tris-HCl (pH7.4), ThermoFisher Scientific) freshly supplemented with protease inhibitor (Merck Millipore), benzonase (Merck Millipore), 5 mM DTT (Sigma-Aldrich), and 1% PhosSTOP™ phosphatase inhibitor (Roche). Proteins were separated by SDS-PAGE and electro-transferred onto PVDF membranes. Blots were placed in Tris-buffered saline, supplemented with 0.05% Tween (Sigma-Aldrich) and 5% non-fat milk for 1 hour at room temperature and then incubated with primary antibody (diluted in TBS/0.05% Tween) overnight at 4°C while shaking. The following primary antibodies were used: beta-actin (1:5,000 Cell Signaling Technology, #8457), ANK3 (1:1,000 ThermoFisher, #33-8800), AP2M1 (1:1,000 ABCAM, ab106542), MPP2 (1:1,000 ABCAM, ab231634), BAIAP2 (1:1,000 Sigma, HPA023310), SEPT7 (1:1,000 ABCAM, ab175229), CACNB4 (1:1,000 ABCAM, ab85788), ACTN4 (1:1,000 ThermoFisher, #42-1400), ARPC2 (1:1,000 ABCAM, ab133315), HOMER1 (1:1,000 ABCAM, ab88827), EFNB3 (1:1,000 ABCAM, ab101699), RAC1 (1:1,000 ThermoFisher, #PA1-091), NRXN1 (1:1,000 Sigma, SAB4503629), ACTN1 (1:1,000 ABCAM, ab18061), DLGAP2 (1:1,000 ABCAM, ab106520), PRKCZ (1:1,000 Cell Signaling Technology, #9368), MST1 (1:1,000 Cell Signaling Technology, #14946), MOB1 (1:1,000 Cell Signaling Technology, #13730), pMOB1 (Thr^{35}) (1:1,000 Cell Signaling Technology, #8699), LATS1 (1:1,000 Cell Signaling Technology, #3477), pLATS1/2 (Thr1079/1042) (1:1,000 Cell Signaling Technology, #8654), LATS2 (1:1,000 Cell Signaling Technology, #5888), KIBRA (1:1,000 Cell Signaling Technology, #8774), GLUA1 (1:1,000 Cell Signaling Technology, #13185), GLUA2 (1:1,000 Cell Signaling Technology, #5306), GLUA3 (1:1,000 Cell Signaling Technology, #4676), CACNG8/ TARPγ-8 (1:1,000 ABCAM, ab116142), HA (1:8,000 Cell Signaling Technology, #3724), FLAG (1:1,000 Sigma, F3165), PICK1 (1:1,000 Cell Signaling Technology, #85325), GRIP1 (1:1,000 ThermoFisher, #PA5-67825), SAP97 (1:1,000 ThermoFisher, #PA1-741), PSD95 (1:1,000 Cell Signaling Technology, #2507), CASK (1:1,000 Cell Signaling Technology, #2878), PACSIN (1:1,000 Millipore, AB10439), SHISA6 (1:1,1000 Novus Biologicals NBP1-93747), DNM1 (1:1,000 Sigma SAB2100611), SYNPO (1:1,000 ThermoFisher, #PA5-21062), YWHAB (1:1,000 ThermoFisher, #PA1-37002), AKAP5 (1:1,000 ThermoFisher, #PA5-36155), NCAM1 (1:1,000 Cell Signaling Technology, #99746), MYO6 (1:1,000 ThermoFisher, #PA5-68238), CAMKIIα (1:1,000 ThermoFisher, #MA1-048), KIF5B (1:1,000 Cell Signaling Technology, #18148), DNAI1 (1:1,000 Sigma HPA021843), Secondary antibodies: anti-rabbit IgG, HRP-linked antibody (1:10,000, Cell Signaling Technology, #7074), anti-mouse IgG, HRP-linked antibody (1:10,000, Cell Signaling Technology, #7076).

Subsequently, blots were washed and probed with the respective horseradish peroxidase- (or fluorophore-conjugated) secondary antibody for 1 hour at room temperature. The reactive bands were visualized using ECL detection reagent (BioRad). Determination of the band intensities were performed with BioRad, ChemiDoc MP.

In general, protein quantification was performed by normalization to the intensity of actin, which was determined on the same membrane. For quantification of phosphorylated proteins this signal was always referred to the signal intensity of the corresponding total protein.

Transfections

Primary hippocampal neurons were either transfected using adeno-associated viruses (AAVs) or by nucleofection. For nucleofection cells (2×10^6) were resuspended in 100 µL of transfection buffer (50 mM HEPES (pH 7.3), 90 mM NaCl, 5 mM KCl, and 0.15 mM $CaCl_2$). Up to 2 µg of plasmid DNA was added to the cell suspension, and electroporation was carried out using the Amaxa 2B-Nucleofector system (Lonza). Cells were replated at a density of 10^5 cells/cm^2.

Co-immunoprecipitation (Co-IP)

Immunoprecipitations of tagged or untagged proteins from protein extracts were performed. Briefly, 500 µg of lysate was incubated overnight with 2 µg of the appropriate IP-antibody HA (Cell Signaling Technologies, #3724) or IgG as control; FLAG (Sigma, F3165); and LATS2 (Cell Signaling Technologies, #5888) or GLUA1 (Cell Signaling Technology, #13185) at 4°C. 20 µL of protein G dynabeads (Invitrogen, 100-03D) was blocked with bovine serum albumin and subsequently added to the lysate-antibody mix and allowed to incubate at 4°C for 3 h in order to mediate binding between the dynabeads and the antibody-antigen complex of interest. The beads were washed three times with ice-cold PBS and were eluted as follows for (WB). The protein-antibody complexes were eluted with 60 µL Laemmli loading buffer. Thereafter, the eluate was boiled for 5 min at 95°C. Then 2-5 µL of each immunoprecipitated reaction product was separated by SDS-PAGE and electro-transferred onto nitrocellulose membranes.

Quantitative PCR (RT-qPCR) analysis

Mice were treated with MSTi (3 mg kg^{-1}; i.p., all in vivo experiments) and subsequently, animals were anesthetized (Isofluran CP®, cp-pharma) and decapitated, organs were dissected and stored on dry ice immediately. Total RNA was isolated from brain (hippocampus, prefrontal cortex) and peripheral tissue (liver) using the Rneasy Mini Kit (Qiagen, 74104). 5 µg of total RNA were reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher, 4368814). Quantitative PCRs were performed using the TaqMan StepOnePlus Real-Time PCR System and a TaqMan 5′-nuclease probe method (Thermo Fisher). All transcripts were normalized to *Hprt* and *Gapdh*. Predesigned human TaqMan assays (Thermo Fisher) were used for quantifying gene expression of *Ctgf* (Mm01192933_g1) *Cyr61* (Mm00487498_m1), *Hprt* (Mm03024075_m1), and *Gapdh* (Mm99999915_g1).

Kinome

For the analysis of MSTi targets in SH-SY5Y cells, kinobead γ pulldowns were performed as previously described (*35, 64*). Briefly, cells were lysed in 0.8% NP40, 50 mM Tris-HCl pH 7.5, 5% glycerol, 1.5 mM MgCl2, 150 mM NaCl, 1 mM Na3VO4, 25 mM NaF, 1 mM DTT, protease inhibitors (SigmaFast, Sigma) and phosphatase inhibitors. MSTi was spiked into 1 mL of lysate, which was adjusted to 5 mg/mL protein, with increasing concentrations (DMSO control, 3 nM, 10 nM, 30 nM, 100 nM, 300 nM, 1 µM, 3 µM and 10 µM) and incubated for 45 min at 4°C. Afterwards, incubation with 35 µL settled kinobeads took place for 30 min at 4°C. In order to enable the determination of a correction factor for each protein and to calculate apparent dissociation constants, a second kinobead pulldown (with fresh beads) was performed on the unbound fraction of the DMSO control. Proteins bound to kinobeads were eluted with LDS sample

buffer (NuPAGE, Thermo Fisher Scientific) containing 50 mM DTT and alkylated with 55 mM CAA. Kinobead pulldown eluates were purified by short SDS-PAGE (ca. 1 cm; Thermo Fisher Scientific) and were subsequently subjected to in-gel tryptic digestion according to standard procedures. After drying in a centrifugal evaporator, the samples were stored at -20°C until LC-MS/MS analysis.

LC-MS/MS measurement of peptides in eluates was performed using an UltiMate 3000 (Thermo Fisher Scientific) coupled to a quadrupole-Orbitrap Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific). Peptides were separated on an Acclaim PepMap analytical column (0.1 mm x 15 cm, C18, 2 µM, 100 Å; Thermo Fisher Scientific) using a 15 min linear gradient from 3-28% solvent B (0.1% formic acid, 5% DMSO in acetonitrile) in solvent A (0. 1% formic acid, 5% DMSO in water) at a flow rate of 50 µL min^{-1}. The mass spectrometer was operated in data dependent acquisition and positive ionization mode. MS1 spectra were acquired over a range of 360-1300 m/z at a resolution of 60,000 in the Orbitrap by applying an AGC of 3 x e^6 or maximum injection time of 50 ms. Up to 12 peptide precursors were selected for fragmentation by higher energy collision-induced dissociation (HCD; 1.3 m/z isolation window, AGC value of 1 x e^5, maximum injection time of 22 ms) using 28% normalized collision energy (NCE) and analyzed at a resolution of 15,000 in the Orbitrap. Protein and peptide identification and quantification was performed using MaxQuant (version 1.6.3.3) (*63*) by searching the tandem MS data against all human canonical and isoform sequences as annotated in the Swissprot reference database (42355 entries, downloaded 06.06.2018) using the embedded search engine Andromeda (*65*). Carbamidomethylated cysteine was set as fixed modification and oxidation of methionine and N-terminal protein acetylation as variable modification. In addition, phosphorylation of serine, threonine and tyrosine was set as variable modification. Trypsin/P was specified as the proteolytic enzyme and up to two missed cleavage sites were allowed. Precursor tolerance was set to 4.5 ppm and fragment ion tolerance to 20 ppm. The minimum peptide length was set to seven and all data were adjusted to 1% propensity score matching (PSM) and 1% protein false discovery rate (FDR). A minimum score for modified peptides was set to 40. Label-free quantification (*66*) and data matching were enabled within MaxQuant. Protein intensities were normalized to the respective DMSO control. IC50 and EC50 values were deduced by a four-parameter log-logistic regression using an internal pipeline that utilizes the 'drc' package in R. A Kdapp was calculated by multiplying the estimated EC50 with a protein-dependent correction factor that was limited to a maximum value of 1. The correction factor for a protein is defined as the ratio of the amount of protein captured from two consecutive pulldowns of the same DMSO control lysate (*67*) and was set to the average of correction factors across all experiments using the same lysate and beads.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (*68*) partner repository with the dataset identifier PXD050351.

Voltage-clamp electrophysiology in vitro

All experiments were carried out at room temperature. In the submerged-type recording chamber, slices were continuously superfused with carbogenated physiological saline (5–6 ml min^{-1} flow rate. Recordings were made under an upright microscope (SliceScope Pro 6000, Scientifica) equipped with infrared videomicroscopy and a gradient contrast system to visualize individual neurons in area CA1. Somatic whole-cell patch-clamp recordings from CA1 pyramidal cells (>1 GΩ seal resistance) were performed in voltage-clamp mode (−70 mV holding potential) using an EPC 10 intracellular amplifier (Heka). The current was low-pass filtered at 1.3 kHz and digitized at 6.5 kHz. The patch-clamp electrodes (5–7 MΩ open-tip resistance) were pulled from borosilicate

glass capillaries (Harvard Apparatus) and filled with a solution (approximately adjusted to pH 7.4) consisting of (in mM): 130 K-gluconate, 5 NaCl, 2 $MgCl_2$, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.3 Na-GTP, 20 phosphocreatine, and 5 D-glucose (potentials were corrected for a liquid junction potential of 12 mV). The extracellular solution additionally contained tetrodotoxin (1 µM, Alomone Labs), bicuculline methiodide (10 µM, Tocris) and D-APV (100 µM, Tocris). The access resistance (*R*a) was continuously monitored. Recordings were terminated if *R*a changed > 10%. MSTi (3 µM) was bath applied to slices if baseline recording was stable over 10 min.

Field potential recordings in vitro

All experiments were carried out at room temperature. In the submerged- type recording chamber, slices were continuously superfused with carbogenated ACSF (5–6 ml min^{-1} flow rate). Field potentials in the CA1 stratum pyramidale were recorded using glass microelectrodes (~1 MΩ open-tip resistance), pulled from borosilicate glass capillaries (Harvard Apparatus), filled with ACSF, that were connected to an extracellular amplifier (EXT-01, npi electronic). Recording data were low-pass filtered at 1 kHz and digitized at 5 kHz. LTP was induced (100 Hz for 1 s) or MSTi was bath applied to slices if baseline recording was stable over 20 min.

Preparation and staining of brain slices for voltage-sensitive dye imaging (VSDI)

Mice were anesthetized with isoflurane and decapitated. All following steps were done in ice-cold sucrose- based saline saturated with carbogen gas (95%/5% O_2/CO_2). This saline (approximately adjusted to pH 7.4) consisted of (in mM): 87 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 7 $MgCl_2$, 25 D-glucose, and 75 sucrose. The hemispheres were prepared for the slicing procedure by a special transversal cut, which is sometimes called "magic cut" (*69*), to conserve the intrahippocampal axonal projections along the hippocampal longitudinal axis. Subsequently, 350 µm-thick horizontal slices were cut using a vibratome (HM650V, Thermo Scientific). Slices were incubated in carbogenated sucrose-based saline for 30 min at 34 C. Subsequent staining with the voltage-sensitive dye Di-4-ANEPPS (Sigma-Aldrich; dissolved in DMSO to a 20.8 mM stock solution) was carried out at room temperature (23–25°C). Slices were kept for 15 min in carbogenated ACSF containing Di-4-ANEPPS (7.5 µg ml^{-1}; <0.1% DMSO). Afterwards, slices were stored at room temperature for at least 30 min in Di-4-ANEPPS-free carbogenated ACSF containing 0.6 µM bicuculline methiodide (BIM, Tocris) (*44*).

VSDI experiments

VSDI and data analysis were performed using the MiCAM02 hardware and software package (BrainVision). The microscope was equipped with the MiCAM02-HR camera and the 2X and 1X lens at the objective and condensing side, respectively. Acquisition settings were as follows: 88 x 60 pixel frame size, 36.4 x 40.0 µm pixel size, 2.2 ms sampling time, and 440 ms recording time (representing the time period during which the fractional change in fluorescence (ΔF/F) was calculated). Experiments were carried out at room temperature and slices were continuously superfused with BIM (0.6 µM)- containing carbogenated ACSF (4–5 ml/min flow rate). BIM never led to epileptiform activity in the regions of interest. Hippocampal trisynaptic circuit (HTC)-Waves, were evoked by square pulse electrical stimuli (EXT-01, npi electronic, 200 µs pulse width) delivered at 5 Hz using a custom made monopolar tungsten electrode (50 µm tip diameter, ~0.5 MΩ nominal impedance) to the visually identified PP near its entry zone to the DG (fig. S5J). In all slices, PP fibers, which directly innervate CA3 pyramidal cells, were cut at the point where they exit the DG. Temporoammonic projections were likewise functionally inactivated. The intensity of PP stimulation (15-35 V) was adjusted in a manner to produce fast, depolarization-

mediated imaging signals (FDSs) in the DG with amplitudes of 50–80% of the highest attainable value. These FDSs range within the linear upturn of the respective input-output curve. To obtain submaximal HTC-Waves comprising CA1-FDSs with amplitudes of 0.1–0.3% $\Delta F/F$, 7 consecutive stimulation pulses were delivered per recording sequence to the PP (fig. S5J). The number of consecutive stimulation pulses was held constant within an experiment. Acquisitions were made every 2 min and the peak amplitude of the last DG-, CA3-, and CA1- FDS within a recording sequence (fig. S5K) were determined by means of the MiCAM02 software. As final measure of DG, CA3, and CA1 activity, we calculated mean DG-, CA3-, and CA1-FDS amplitude values over three consecutive acquisitions. MSTi was bath applied to slices if baseline recording was stable over 20 min (*44*).

Processing and quantification of VSDI data

All steps were carried out using the MICAM02 software. From VSDI signals, the fractional change in fluorescence ($\Delta F/F$) was calculated and $\Delta F/F$ values were spatially and temporally smoothed using a 3 × 3 × 3 average filter. VSDI signals presented in images were smoothed with a 5 × 5 × 3 average filter. To attenuate slow signal components produced from bleaching of the dye and slight summation of 5 Hz neuronal responses, we afterwards applied a weak high-pass filter (τ = 220 ms) to the imaging data. Pixelation of images was reduced by the interpolation function. For analysis of neuronal population activity in hippocampal subregions, standardized regions of interest (ROIs) were manually set according to anatomical landmarks. The circular CA3-ROI (r = 4 pixels) was positioned into the CA3 region near the DG, but not overlapping with it. The circular CA1-ROI (r = 4 pixels) was placed into the CA1 subfield with a distance of approximately 200 µm from the visually identified distal end of the stratum lucidum. Both ROIs spanned the stratum oriens, stratum pyramidale, and stratum lucidum/radiatum. The DG-ROI, which enclosed the fascia dentata (fig. S5j) was created by the polygon-drawing function. The average of smoothed $\Delta F/F$ values within a particular ROI served as final measure of neuronal population activity (*44*).

Statistical analysis

Behavioral studies: To analyze the development of long-term memory formation in the MWM, we compared the development of escape latencies during the first trial per day over the course of training, using 2-way RM ANOVA, followed by post-hoc test as indicated in the corresponding figure legend. In addition, we compared the mean escape latencies over all training trials (unpaired t-test), and the selectivity of spatial learning by considering the time the animals spent in each quadrant during the probe trial (2-way RM ANOVA, followed by post-hoc test as indicated in the corresponding figure legend. To reveal novel object recognition, we compared the investigation duration of the FO and NO separately per group (two-tailed paired t-tests), and the RIDs (two-tailed unpaired t-tests).

All other data were analyzed by two-tailed paired/unpaired t test or by one-wayANOVA as appropriate using GraphPad Prism software (GraphPad Software, Inc., Version 8). Significant main or interaction effects were followed by Bonferroni or Tukey's post-hoc tests as appropriate. The criterion for significance was set at $P < 0.05$. For all data, results are shown as the mean ± SEM.

Supplementary Materials:

Figures S1–S7

Data files S1–S3

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Figure legends:

Figure 1: Drug-induced segregation of WWC1 from LATS1/2. (A and B) Representative Western blot (WB) of one experiment (A) and quantitative analysis of co-immunoprecipitation (Co-IP) of LATS2-WWC1 complexes, using control IgG or anti-GFP antibody, and lysates from transfected primary hippocampal neurons overexpressing FLAG-LATS2 (wild-type, Thr1042Ala, or Thr1042Glu) and GFP-tagged WWC1 (B). $N = 7$ independent biological replicates per group. **(C)** WBs of Hippo pathway related proteins in hippocampal tissue of mice treated with MSTi (3 mg kg^{-1}) or vehicle. Representative WB of one experiment. **(D)** Quantification of data from (C). $N = 6$ male mice per group. **(E)** Schematic illustration of MSTi-affected kinases using the kinobead assay in SH-SY5Y neuroblastoma cell line. Data represent $N = 10$ concentration-based, independent experiments. **(F)** IP of hemagglutinin (HA)-WWC1 in primary hippocampal neurons treated with MSTi (3 μM) or vehicle. Protein extracts were used for IP of HA-tagged WWC1, using control IgG or anti-HA antibody. Representative WB of one experiment. **(G)** Quantification of data from (F). $N = 4$ independent biological replicates per group. **(H)** Schematic, illustrating the validation of WWC1 interactors from our previously published interactome study (*9*). Co-IP was performed as described in (F) using protein extracts from MSTi- or vehicle treated primary hippocampal neurons, quantified by WB. Scale indicates fold difference of MSTi- compared to vehicle treated neurons. $N = 4$ independent biological replicates per group (Data file S2). Quantification of WBs of pTARPγ-8^{Ser277} over TARPγ-8 in MSTi- vs. vehicle treated primary hippocampal neurons. $N = 4$ independent biological replicates per group. Data are mean ± SEM. $^{\$\$}P < 0.01$ and $^{\$\$\$\$}P < 0.0001$, calculated by one-way ANOVA followed by Bonferroni's post-hoc test (B) and $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$, calculated by two-tailed unpaired *t* tests (D, G and H). WCE = whole cell lysate.

Figure 2: Synaptic WWC1 primes AMPAR regulatory complexes and promotes learning and memory. (A) Mean first trial escape latency in the Morris Water Maze (MWM) over the entire training period (5 days). Mice were treated with MSTi (3 mg kg^{-1}, all in vivo experiments) or vehicle after completion of the daily training with two trials. $N = 12$ male mice per group. **(B)** Escape latencies during the first trial per day over the course of the training for mice in (A). **(C)** Precipitation of biotinylated membrane proteins from extracts of hippocampal slices from male

mice treated with MSTi (3 μM, all in vitro experiments) or vehicle. Representative Western blot (WB) from one experiment. **(D)** Quantification of data from (C). *N* = 12 in the MSTi group and *N* = 11 in the vehicle group. **(E)** Representative traces of miniature excitatory postsynaptic currents (mEPSCs) recorded in hippocampal CA1 pyramidal neurons during baseline with vehicle and in the presence of MSTi. Representative traces from one experiment. Scale bars, 30 pA and 0.5 sec. **(F and G)** Cumulative probability curves and quantification of mEPSCs. *N* = 7 cells from 3 male mice. **(H and I)** Normalised field excitatory postsynaptic potential (fEPSP) slope plus representative field potential (FP) traces recorded during baseline with vehicle and in the presence of MSTi. MSTi was removed from the bath solution after 25 min. *N* = 4 slices from 3 male mice. Representative FP traces from one experiment. Scale bars, 10 ms and 200 μV. **(J)** Normalised fEPSP slope representative FP traces recorded during baseline and after high-frequency stimulation (HFS, 100 Hz for 1 sec.) in slices preincubated with MSTi or vehicle for 20 min. MSTi or vehicle were present during baseline recordings and were removed from the bath solution after HFS. *N* = 5 slices from 4 male mice in the MSTi group and *N* = 7 slices from 4 male mice in the vehicle group. Representative FP traces from one experiment. Scale bars, 10 ms and 200 μV. **(K)** Representative voltage-sensitive-dye imaging (VSDI) filmstrip of a submaximal hippocampal-trisynaptic circuit (HTC) wave evoked by 5 Hz stimulation of the perforant path (PP). Warmer colours represent stronger neuronal activity. Representative images show the average of three electrical stimulations (stimulus 7, sampling time 2.2 ms). Scale bar, 500 μm **(L)** Normalised, fast, depolarization-mediated imaging signal (FDS) amplitudes in the dentate gyrus (DG) and hippocampal areas CA3/CA1 recorded during theta-frequency stimulation of PP fibres plus VSDI images during baseline with vehicle and in the presence of MSTi. *N* = 8 slices from 5 male mice. Representative images show the average of three electrical stimulations. Scale bar, 500 μm. **(M)** Normalised fEPSP slope recorded in *Wwc1*$^{-/-}$ and *Wwc1*$^{+/+}$ mice plus representative FP traces recorded during baseline with vehicle and in the presence of MSTi. *N* = 10 slices from 5 male mice in the *Wwc1*$^{-/-}$ group and *N* = 12 slices from 5 male mice in the *Wwc1*$^{+/+}$ group. Representative FP traces from one experiment. Scale bars, 10 ms and 200 μV. **(N)** Mean first trial escape latency in the MWM over the entire training period (5 days). Male *Wwc1*$^{-/-}$ and *Wwc1*$^{+/+}$ mice were treated with MSTi after completion of the daily training with 2 trials. *N* = 12 male mice in the *Wwc1*$^{-/-}$ group and *N* = 13 mice in the *Wwc1*$^{+/+}$ group. Data are mean ± SEM. $^{\$}p < 0.05$, calculated by two-way RM-ANOVA followed by Bonferroni's post-hoc test (B) and $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$, calculated by two-tailed unpaired *t* tests (A, D, M and N) and two-tailed paired *t* tests (F to G, J, L and M). BL = baseline, WCE = whole cell lysate.

Figure 3: Drug-induced WWC1-LATS1/2 segregation primes AMPAR-dependent cognitive enhancement. (A) Normalised field excitatory postsynaptic potential (fEPSP) slope plus representative field potential (FP) traces recorded during baseline with vehicle and in the presence of MSTi (3 μM). *N* = 10 slices from 3 male mice. Representative FP traces from one experiment. Scale bars, 10 ms and 200 μV. **(B)** Mean first trial escape latency in the Morris Water Maze (MWM) over the entire training period (6 days). 23 months old male mice were treated with MSTi (3 mg kg^{-1}, all in vivo experiments) or vehicle after completion of the daily training with two trials. *N* = 9 in the MSTi group and *N* = 8 mice in the vehicle group. **(C)** Exploration time of a novel object (NO) compared to a familiar object (FO) on day 2. On the training day (day 1), 23-month-old male mice received treatment with MSTi or vehicle, administered 45 min prior to sampling. *N*

= 9 mice in the MSTi group and N = 8 mice in the vehicle group. **(D)** Ratio of investigation duration (RID) for the mice in (C). **(E)** Escape latencies during the first trial per day over the course of the training (9 days). Male *ArcAβ*^{HEMI} and *ArcAβ*$^{++}$ mice were treated with MSTi 45 min before the daily training with two trials. $N = 13$ *ArcAβ*^{HEMI} mice and $N = 15$ *ArcAβ*$^{++}$ mice. **(F)** Exploration time of a NO compared to a FO on day 2. On the training day (day 1), male *ArcAβ*^{HEMI} and *ArcAβ*$^{++}$ mice received treatment with MSTi, administered 45 minutes prior to sampling. $N = 13$ *ArcAβ*^{HEMI} mice and $N = 15$ *ArcAβ*$^{++}$ mice. **(G)** Quantification of pLATS1/2 / LATS2 by immunoblotting from brain protein extracts of 2 months old and 23 months old male mice. $N = 6$ independent biological replicates per group. **(H)** Quantification of pLATS1/2 / LATS2 by immunoblotting of brain protein extracts from male *ArcAβ*^{HEMI} and *ArcAβ*$^{++}$ mice. $N = 6$ independent biological replicates per group. **(I to K)** Quantification of pLATS1/2 / LATS2 (I), WWC1 binding to LATS (J) and WWC1 binding to GLUA1 (K) by capillary immunoblotting in human post-mortem brain protein extracts. $N = 35$ independent biological replicates in the control (CTRL) group and $N = 36$ independent biological replicates in the AD group. **(L)** Precipitation of biotinylated membrane proteins from extracts of brain organoids treated with MSTi or vehicle. Quantification of GLUA1 and GLUA2 by capillary immunoblotting. $N = 4$ independent biological replicates from 4 brain organoids per group. Data are mean ± SEM. $*P < 0.05$, $**P < 0.01$ and $****P < 0.0001$, calculated by two-tailed paired *t* tests (A, C and F), two-tailed unpaired *t* tests (B, D, G to L) and two-way RM-ANOVA followed by Bonferroni's post-hoc test (E).