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The amyloid hypothesis posits that the amyloid-beta (Aβ) protein precedes and requires microtubule-associated protein tau in a sort of trigger-bullet mechanism leading to Alzheimer's disease (AD) pathology. This sequence of events has become dogmatic in the AD field and is used to explain clinical trial failures due to a late start of the intervention when Aβ already activated tau. Here, using a multidisplinary approach combining molecular biological, biochemical, histopathological, electrophysiological and behavioral methods we demonstrated that tau suppression did not protect against Aβ-induced damage of long-term synaptic plasticity and memory, as well as amyloid deposition. Tau suppression could even unravel a defect in basal synaptic transmission in a mouse model of amyloid deposition. Similarly, tau suppression did not protect against exogenous oligomeric tau induced impairment of long-term synaptic plasticity and memory. The protective effect of tau suppression was, in turn, confined to short-term plasticity and memory. Taken together, our data suggest that therapies downstream of Aβ and tau together are more suitable to combat AD than therapies against one or the other alone.

TITLE

Tau is not necessary for amyloid-beta-induced synaptic and memory impairments

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ABSTRACT

The amyloid hypothesis posits that the amyloid-beta (Aβ) protein precedes and requires microtubule-associated protein tau in a sort of trigger-bullet mechanism leading to Alzheimer's disease (AD) pathology. This sequence of events has become dogmatic in the AD field and is used to explain clinical trial failures due to a late start of the intervention when Aβ already activated tau. Here, using a multidisplinary approach combining molecular biological, biochemical, histopathological, electrophysiological and behavioral methods we demonstrated that tau suppression did not protect against Aβ-induced damage of long-term synaptic plasticity and memory, as well as amyloid deposition. Tau suppression could even unravel a defect in basal synaptic transmission in a mouse model of amyloid deposition. Similarly, tau suppression did not protect against exogenous oligomeric tau induced impairment of long-term synaptic plasticity and memory. The protective effect of tau suppression was, in turn, confined to short-term plasticity and memory. Taken together, our data suggest that therapies downstream of Aβ and tau together are more suitable to combat AD than therapies against one or the other alone.

Introduction

The “Amyloid Cascade” hypothesis dominates in the Alzheimer’s disease (AD) field. It posits that amyloid-β (Aβ) and tau proteins are placed in series with Aβ upstream of tau, in a sort of trigger-bullet mechanism. In support of this hypothesis, a decrease or genetic suppression of tau prevents Aβ-induced synaptic damage, neuronal toxicity, and axonal defects (1-3). Moreover, reducing tau levels counteracts Aβ-induced synaptic plasticity and behavioral abnormalities (1, 4, 5), as well as spreading of the pathology throughout the brain (6). Consistent with these findings, the expression of human wild type, but not mutant N296H tau, rescues the Aβ-induced inhibition of LTP in tau knock-out (KO) mice (7). As a consequence of the amyloid cascade hypothesis, many scientists have ascribed the failure of anti-Aβ clinical trials to a late intervention in the disease development when Aβ has already triggered tau, producing pathology independently of Aβ. Indeed, a large number of studies are currently focusing either on starting anti-Aβ therapies early in the disease progression when tau has not yet been triggered by Aβ, or alternatively, on the role of tau in AD pathogenesis, with the ultimate goal of arresting the disease by acting onto tau.

Numerous studies suggest that Aβ and tau have a common toxicity mechanism. Both proteins i) are released upon neuronal activity (8-13), ii) permeate neuronal and glial cells (14-20), iii) undergo spreading throughout the brain (21), iv) impair synaptic function and memory (10, 22, 23), and v) need cellular prion protein for disrupting hippocampal synaptic plasticity (24). Most importantly, Aβ and tau cooperate to produce behavioral deficits,

synaptic dysfunction, and downregulation of transcription of genes involved in synaptic function (10, 25). Interestingly, concurrent administration of low sub-toxic doses of oligomers of Aβ (oAβ) and tau (oTau) produces an immediate disruption of memory and hippocampal long-term potentiation (LTP), a type of synaptic plasticity thought to underlie memory formation (10), supporting the idea that the two proteins might act in parallel to exert their detrimental effects (26). Consistent with this idea, amyloid precursor protein (APP) is necessary for the detrimental effect of Aβ and tau onto LTP and memory (19) with both Aβ and tau binding APP (19, 27-31). Taken all together, these results beg the question of whether Aβ and tau are placed in series or in parallel in the processes leading to synaptic dysfunction and memory loss. Addressing this question is of high relevance in the field because if the two proteins act in parallel, both anti-Aβ and anti-tau therapies alone are doomed to fail. Solving this conundrum is of paramount relevance for the design of anti-AD clinical trials.

Here, we demonstrate that tau is not necessary for the Aβ-induced impairment of long-term synaptic plasticity and memory, as well as for amyloid deposition. Tau suppression can even unveil a deficit in basal neurotransmission in amyloid deposting mice. The role of tau in Aβ-induced damage would be restricted only to the impairment of short-term synaptic plasticity and memory.

Results

Endogenous tau expression is not required for disruption of long-term synaptic plasticity and memory induced by overexpression of mutated APP. To provide an in-depth analysis of the relationship between Aβ and tau, we tested the effects of knocking out tau expression onto LTP reduction by mutated APP overexpression. To this end, we crossed *Mapt-KO* mice (32) with transgenic mice overexpressing human APP carrying the Swedish (APP KM670/671NL) and the Indiana (V717F) mutations (named TgAPP) (33). APP expression in these transgenics is driven by the neuron-specific prion promoter to generate a model of AD-related amyloid pathology where Aβ depositions are observed at 3-4 months of age (33). Interestingly, we found that basal synaptic transmission was impaired in 9-12 month old TgAPP*/Mapt*-KO mice compared to TgAPP, *Mapt*-KO and WT littermates (Figure 1A), suggesting that the combination of mutated APP overexpression with tau suppression is deleterious to basal neurotransmission. LTP analysis in slices from TgAPP*/Mapt*-KO and TgAPP mice revealed an impairment at 120 min after the theta-burst compared to WT or *Mapt*-KO littermates (Figure 1B,C), whereas the impairment was not present in TgAPP*/Mapt*-KO mice at 30 min after tetanus (Figure 1B,C). These findings show that tau suppression unveils a defect in basal neurotransmission in mice overexpressing mutated APP. Additionally, mutated APP overexpression with chronic expression and accumulation of naturally produced Aβ affects long-term plasticity despite the absence of endogenous tau. The role of tau was confined to the short-term phase of LTP.

Given that LTP is a cellular correlate of memory, we evaluated cognitive function in TgAPP*/Map*t-KO mice compared to the other groups. Analysis of spatial memory through the 2-day RAWM showed an impairment in TgAPP and TgAPP*/Mapt*-KO mice which made more errors than WT and *Mapt*-KO littermates (Figure 1D), suggesting decreased spatial memory in both animal models. Most importantly, tau suppression did not protect against the spatial memory damage in animals overexpressing mutated APP.

We obtained consistent results when we examined contextual fear memory after an electric shock. The amount of freezing in TgAPP and TgAPP*/Mapt*-KO mice was lower than in WT and *Mapt*-KO littermates when the animals were exposed to the same context at 24 hrs after training (Figure 1E), suggesting that tau suppression does not protect against the impairment of contextual fear memory in mice overexpressing mutated APP.

Given that electrophysiological experiments showed that tau suppression protects against the damage of the initial phase of LTP in TgAPP*/Mapt*-KO mice, we wondered whether endogenous tau, although not required for the APP overexpression induced impairment of long-term memory, blocks the effect of the overexpression onto short-term memory. To this end, we used fear conditioning that allows analyzing learning at specific time intervals after training. Evaluation of contextual fear learning at 30 min after training showed that TgAPP*/Mapt*-KO mice presented similar freezing as WT littermates (Figure 1F), suggesting that the short-term memory defect by mutated APP overexpression is rescued by tau suppression.

Analysis of amygdala-dependent cued memory at 24 hrs after examination of contextual fear memory was also interesting. As previously shown (34, 35), it revealed an impairment of cued memory in TgAPP mice compared to WT mice. Interestingly, the defect was not rescued by tau suppression in TgAPP/*Mapt*-KO mice (Figure 1G), suggesting that tau suppression will not rescue the defect in emotional memory of AD patients.

No differences among genotypes were found in animal capability of perceiving the electric shock as measured in sensory threshold assessment (Supplemental Figure 1A). Moreover, time and speed to reach a visible platform above the surface of the water (Supplemental Figure 1B,C), and locomotor activity and anxiety-like behavior in an open field task (Supplemental Figure 1D,E) were not affected, suggesting that differences among mice with different genotypes did not cause any sensorial, motor, or motivational defects that might produce the observed effects with the RAWM and contextual fear memory tests. Overall, these experiments suggest that tau suppression protects against the detrimental effects of Aβ on synaptic function and memory only on short-term, but not long-term memory.

We then analyzed amyloid load in TgAPP/*Mapt*-KO mice and TgAPP littermates. As previously shown on the J20 mice in a Mapt-KO background (1), this assessment did not reveal any difference between the two genotypes (Figure 1H). This finding supports the observation that Aβ-induced synaptic plasticity and memory loss are independent of tau suppression. Most

importantly, it extends to AD histopathology the concept that tau suppression is not beneficial against Aβ-induced AD progression.

Endogenous tau expression is not required for disruption of long-term synaptic plasticity and memory induced by oAβ exposure. APP overexpression could affect neuronal function through a number of different mechanisms such as overproduction of APP itself or different fragments of its processing, including Aβ. Thus, to further investigate the relationship between Aβ and tau and determine the role of Aβ in the observed long-term synaptic plasticity and memory defects, we studied LTP following oAβ administration (Figure 2A) in brain slices from 4-6 month old *Mapt*-KO mice. In preliminary experiments, we compared basal synaptic transmission in slices from *Mapt*-KO mice and WT littermates and found a similar input/output relationship (Figure 2B). Next, we confirmed previous findings (36) showing that a brief, 20 min, perfusion with a preparation containing synthetic oAβ (200 nM) before a theta-burst stimulation impairs hippocampal LTP at the CA3-CA1 synapse in slices from WT mice both at a short time after induction of potentiation (30 min) and at a later time point (120 min) (Figure 2C,D). However, oAβ behaved differently on slices from *Mapt*-KO mice. It was able to impair the late phase of LTP at 120 min after the theta burst, whereas no impairment was present at 30 min after the tetanus (Figure 2C,D). Importanly, application of oAβ did not affect basal neurotransmission, as shown by lack of drifting of the baseline (Figure 2C) and similar input/output relationship in slices from *Mapt*-KO and WT mice treated with either vehicle or oAβ (Supplemental Figure 2A). These findings strongly support the hypothesis that the role of endogenous tau in

Aβ-induced impairment of LTP is confined to the early phase without affecting the late phase of LTP.

We then investigated the effects of tau suppression on oAβ-induced memory loss in *Mapt-KO* mice. As previously demonstrated (19), a brief, 60 sec, infusion of a preparation containing synthetic oAβ into dorsal hippocampi (200 nM, in 1 µl bilaterally, 20 min prior to the 1st and 7th trial on both days of the 2-day RAWM task) increased the number of errors with the 2-day RAWM in both WT and *Mapt*-KO mice compared to vehicle-treated littermates (Figure 2E), suggesting decreased spatial memory after Aβ treatment regardless of endogenous tau suppression.

We obtained consistent results when we bilaterally infused the same preparation (200 nM, over 60 sec) into the hippocampi 20 min prior to the electric shock to induce fear conditioning. The amount of freezing in oAβ-treated WT and *Mapt*-KO littermates was lower than in vehicle-treated siblings when the animals were exposed to the same context at 24 hrs after training (Figure 2F), suggesting an impairment of contextual fear memory. Thus, endogenous tau is not needed for oAβ to impair long-term memory.

Electrophysiological experiments showed that tau suppression protects against oAβ damage of the initial phase of LTP. Thus, we wondered whether endogenous tau, although not required for the oAβ-induced impairment of long-term memory, could block the effect of oAβ onto short-term memory. Evaluation of contextual fear learning at 30 min after training showed that

Mapt-KO mice infused with 200 nM oAβ at 20 min before training did not exhibit a significant reduction of freezing compared to vehicle-treated WT mice (Figure 2G), suggesting a protection against Aβ-induced short-term memory loss in *Mapt*-KO mice.

The Aβ-induced defects observed with the RAWM and contextual fear conditioning could be attributed to hippocampal impairment because in control experiments we did not find any differences in cued memory among the four groups of mice (Figure 2H), suggesting no amygdala involvement in the effects of Aβ in fear memory. Moreover, oAβ did not modify animal capability of perceiving the electric shock as measured in sensory threshold assessment (Supplemental Figure 2B), time and speed to reach a visible platform above the surface of the water (Supplemental Figure 2C,D), or locomotor activity and anxiety-like behavior in an open field task (Supplemental Figure 2E,F), suggesting that Aβ did not cause any sensorial, motor, or motivation defects that might have been responsible for its effects on RAWM and contextual fear memory tests.

Exogenously applied oTau impairs long-term synaptic plasticity and memory regardless of endogenous tau suppression. Similar to oAβ (23), oTau impairs both hippocampal LTP and memory (10, 22, 23). Both peptides share APP as a molecule necessary to reduce LTP and memory (19). We therefore investigated the relationship between tau oligomers (Figure 3A) and tau itself with respect to the impairment of LTP and memory, by supplementing synapses with oTau in the absence of endogenous tau. Recording of basal

synaptic transmission in slices from 4-6 month old *Mapt*-KO and WT littermate mice confirmed the lack of differences between the two groups already shown in Figures 1A and 2B (Figure 3B). Slices perfused for 20 min with 50 nM oTau prior to inducing LTP revealed a marked reduction of potentiation at 120 min after the tetanus in slices from *Mapt*-KO and WT mice compared to vehicle-treated slices (Figure 3C,D). Conversely, no differences between oTau- and vehicle-treated slices were observed when analyzing the initial phase of LTP in slices from *Mapt*-KO mice (Figure 3C,D). In addition, oTau did not affect basal neurotransmission, as shown by lack of drifting of the baseline (Figure 3C) and similar input/output relationship in slices from *Mapt*-KO and WT mice treated with either vehicle or oTau (Supplemental Figure 3A). Collectively, these results suggest that endogenous tau is not needed for the oTau-induced impairment of the late phase of LTP whereas it is needed in the initial phase of LTP.

To examine the relevance of human tau onto memory regardless of suppression of endogenous murine tau, we administered oTau through cannulas into the dorsal hippocampi (500 nM, in 1 µl bilaterally, 20 and 180 min prior to the 1st trial of both days of the 2-day RAWM task, over 60 sec). Infusion of oTau revealed a higher number of errors both in the WT and *Mapt*-KO mice (Figure 3E). Moreover, tau suppression did not protect mice against the damage of contextual fear memory induced by oTau (500 nM, bilaterally, 20 and 180 min prior to the electric shock, over 60 sec) at 24 hrs after the electric shock (Figure 3F). Similar to oAβ, the protection was instead present when memory was assessed at 30 min (Figure 3G). Finally, we did not

observe any behavioral differences between various groups of mice when they were tested for cued conditioning (Figure 3H), sensory threshold (Supplemental Figure 3B), visible platform (Supplemental Figure 3C,D) and open field (Supplemental Figure 3E,F). Taken together, these data demonstrate that similar to oAβ, tau suppression does not protect against the detrimental effects of oTau on long-term synaptic plasticity and memory.

Blockage of soluble guanylyl cyclase (sGC) abolishes the protective effect of tau suppression against oAβ- or oTau-induced defect in short-term synaptic plasticity. The early phase of LTP depends upon the nitric oxide/cGMP signaling pathway (37). For instance, 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ), an inhibitor of sGC, the enzyme that produces cGMP, reduces the early phase of LTP (38, 39). To provide insight into the molecular mechanism by which loss of tau confers protection against reduction in short-term plasticity, we examined whether inhibiting sGC blocks the rescue of short-term plasticity by tau suppression. Basal synaptic transmission was similar in slices from 4-6 month old *Mapt*-KO and WT littermate mice (Figure 4A), confirming observations in Figures 1A, 2B, and 3B. As previously demonstrated (40), ODQ perfusion (10 μM, for 10 min prior to the theta-burst) dramatically reduced LTP in WT slices (Figure 4B). A similar reduction in potentiation was present in *Mapt*-KO slices treated with the inhibitor (Figure 4C). As shown in Figures 2C-D and 3C-D, both oAβ and oTau were not capable of impairing the early phase of LTP in *Mapt*-KO slices (Figure 4C). However, ODQ perfusion unveiled a defect of LTP at 30 min after the tetanus in *Mapt*-KO slices treated with either oAβ or oTau (Figure 4C-D). Finally, ODQ

did not further depress LTP in oAβ- or oTau-treated WT slices (Figure 4B and D). The above findings suggest that disruption of cGMP signaling reverses the neuroprotective action of endogenous tau suppression against oAβ- or oTau-induced impairments of the early phase of synaptic plasticity.

Overexpression of wild-type human tau abolishes the protective effect of tau suppression against oAβ-induced short-term defects in LTP and memory. The protection against the negative effects of Aβ onto the initial phase of LTP and short-term memory in *Mapt*-KO mice might not be specific to altered tau expression. To determine specificity of the effect, we overexpressed human wild-type 4R/2N tau in *Mapt*-KO mice by generating htau/*Mapt*-KO mice. The mice express wild-type, full-length oligomer prone human tau (2N4R htau) using the prion cos-tet promoter which results in a largely neuronal expression of the transgene (41) (Figure 5A and Supplemental Figure 4A). These mice display human oTau at 8 months of age (Figure 5B) when TOC1 positive oTau levels were equal to ~0.5 nM, and both LTP and memory impairments were not yet present given that they appeared only after 10 months of age (Supplemental Figure 4B-F). Analysis of basal synaptic transmission at 6-8 months confirmed normal neurotransmission in slices from htau/*Mapt*-KO compared to those derived from both *Mapt*-KO and WT littermates (Figure 5C). Moreover, LTP was normal compared to *Mapt*-KO and WT littermates (Figure 5D). However, when slices from htau/*Mapt*-KO were perfused with subtoxic doses (50 nM) of oAβ prior to the tetanus, they exhibited reduced LTP both at 30 min and 120 min after the tetanus, whereas this concentration of Aβ was not sufficient to disrupt plasticity in slices from WT or *Mapt*-KO

littermates (Figure 5D,E), presumably because 50 nM Aβ concentration is subthreshold for LTP impairment (42, 43).

Likewise, an oAβ concentration subthreshold for memory impairment, 75 nM (42), impaired spatial memory (Figure 5F) and contextual fear learning in htau/*Mapt*-KO mice, but not in *Mapt*-KO and WT mice (Figure 5G,H). The impairment of contextual memory was present both at 30 min and 24 hrs after the electric shock (Figure 5G,H). No differences were found in cued conditioning (Figure 5I), sensory threshold (Supplemental Figure 5A), visible platform (Supplemental Figure 5B,C) and open field (Supplemental Figure 5D,E) among groups of mice. Overall, these experiments demonstrate that the protective effect of tau suppression against the Aβ-induced reduction of the initial phase of LTP and short-term memory is specific to tau.

Discussion

The prevailing hypothesis in AD research is that Aβ precedes tau in causing pathology. Tau would mediate, or accelerate, the pathogenic effects of Aβ (44). Such a hierarchical profile in the chain of events leading to memory loss in AD is used as an explanation for the failures of many clinical trials, mostly targeting Aβ. Two types of strategies are currently being implemented to overcome this obstacle. In one line of research, anti-Aβ therapies are being administered prior to the overt disease manifestation. In the other, various aspects of tau pathology, including tau post-translational modifications, or tau levels, or tau aggregation status are being targeted. However, this model was recently challenged by studies suggesting that Aβ and tau act in parallel instead of being in series (10, 19, 24, 25). In this manuscript, we demonstrate that neither exogenous oAβ nor oTau need endogenous mouse tau to negatively impact the late phase of CA3-CA1 LTP and long-term hippocampal memory. Moreover, we find that tau suppression does not reduce amyloid load in a mouse model of amyloid deposition, and even unravels a defect in basal neurotransmission in the model. These evidences suggest that, at least for certain electrophysiological, behavioral, and histopathological aspects, Aβ and tau act in parallel, and not in series as the amyloid cascade hypothesis predicts.

Tau suppression was shown to protect against synaptic plasticity and memory defects in transgenic mice overexpressing mutated forms of APP (1, 45, 46). However, we found that TgAPP/*Mapt*-KO mice display abnormal synaptic plasticity and memory. Different results between our experiments and earlier

investigations might reflect the different experimental paradigm used among studies. For instance, previous investigations examined LTP at the medial perforant path synapse with the *dentate gyrus* and followed it for 60 min (46). In contrast, we have investigated the CA3-CA1 synapse for 2 hrs. Additionally, previous studies used either the Morris water maze or the T-maze (1, 45, 46), while we used the RAWM and fear conditioning. Additionally, different APP and tau-KO models were utilized (APP models: the CRND8 mouse in our experiments compared to the APP23 and the J20 in the other studies; tau-KO models: the Jackson 007251 strain (32) in which the mouse tau gene was functionally disrupted by replacing exon 1 with the neomycin resistance cassette in our experiments and Roberson's experiments compared to mice in which tau expression was disrupted through insertion of the EGFP cDNA into exon 1 of the tau locus *Mapt* in the Ittner studies (45). Regardless, these results strongly support the hypothesis that the tau-dependence for the effects of oAβ is confined to certain aspects of the pathology, but not necessarily to all aspects of the disease including the late phase of LTP, long-term memory, basal neurotransmission, or amyloid deposition. Consistent with this conclusion, similar to cultured hippocampal wild-type neurons, tau-KO neurons show a reduction of axonal transport after oAβ exposure, indicating that tau is not required for transport disruption (47), and likewise tau can impair axonal transport independent of Aβ (48).

The early phase of LTP is protein synthesis independent whereas its late phase is protein synthesis dependent and requires gene transcription (49). Indeed, protein synthesis inhibitors do not prevent learning of tasks but disrupt

memory of the training (50), supporting the view that there are different stages of memory with an early protein-synthesis independent stage and a late protein-synthesis dependent one that is required for consolidation of long-term memories (51). Based on this view and considering findings in the current manuscript including protection against oAβ- and oTau-induced impairments by tau suppression of short-term forms of plasticity and memory but not the long-term forms, we predict that although AD patients might respond to learning training after tau suppression, they may remain unable to consolidate memories.

Despite the main finding of our studies is lack of protection against damage of late phases of synaptic plasticity and long-term memory by tau suppression, we also interrogated the molecular basis of protection against the short-term plasticity defect by tau suppression. We found that inhibition of cGMP signaling via the sGC inhibitor ODQ blocks the neuroprotective effect of endogenous tau suppression against oAβ- and oTau-induced impairments of the early phase of synaptic plasticity. Moreover, the inhibitor did not further depress short-term plasticity in WT slices incubated with either oAβ or oTau, suggesting that oAβ and oTau impair plasticity via cGMP inhibition and not through additional independent mechanisms. These findings are consistent with the observation that the early phase of LTP requires an intact nitric oxide/cGMP signaling (37-39). Interestingly, both oAβ and oTau modulate hippocampal cGMP levels after LTP or memory induction (36, 40). Thus, it is likely that the protection against the oAβ- and oTau-induced defects of the early-phase of LTP in *Mapt*-KO mice is linked with cGMP signaling.

We found a reduction in basal synaptic transmission of TgAPP/*Mapt*-KO mice compared to the other groups including the TgAPP mice. The analysis of neurotransmission in different transgenic models overexpressing mutated APP has often shown an impairment of basal neurotransmission at later stages than the LTP impairment (52, 53). Considering that tau performs multiple physiological functions (54), it is possible that tau suppression might exacerbate the negative effect of mutated APP overexpression onto basal neurotransmission. Independent of the impacts on basal synaptic transmission, this raises an additional concern against the possibility of utilizing tau suppression therapies (albeit given that the reduction of basal synaptic transmission was observed in animals overexpressing mutated APP in a complete absence of tau, one cannot conclude that a partial tau suppression such as the one obtained with tau antisense oligonucleotides or antibodies would definitively impair synaptic function in AD patients).

Similar to long-term contextual fear memory, we did not find a rescue of the cued fear memory impairment following tau suppression in TgAPP/*Mapt*-KO mice. Cued fear conditioning is an amygdala dependent and hippocampus-independent task (55). Interestingly, the amygdala is affected both in AD mouse models and AD patients (56). It characteristically shows shrinkage, distortion and loss of neurons, and widespread gliosis in AD patients (57-59). Moreover, emotional memory impairment in AD patients positively correlates with amygdala atrophy (56). Altogether, these findings suggest that tau suppression will not rescue the defect in emotional memory of AD patients.

Consistent with the findings on LTP and memory, analysis of amyloid load did not show any difference between TgAPP*/Mapt*-KO and TgAPP mice. These results were similar to studies in J20 mice crossed with *Mapt*-KO animals (1), supporting the observation that Aβ-induced synaptic plasticity and memory loss are independent of tau suppression. Most importantly, they extend to AD histopathology the concept that tau suppression is not beneficial against AD progression.

Another finding in our studies is that exogenously applied oTau impairs the late phase of LTP and long-term memory regardless of endogenous tau suppression. This is interesting because it suggests that oTau behaves similar to oAβ. Consistent with this conclusion, the two proteins share several biochemical, physiological, and pathological features in common (26). Both are involved in synaptic plasticity in the normal healthy brain (60, 61), whereas in the diseased brain they form toxic oligomeric species, probably because they form β-sheets (10, 62-64). Moreover, sub-toxic doses of oTau and oAβ produce coordinated changes in synaptic plasticity and memory (10). Most importantly, this finding highlights differences in the mechanism of action of tau in its native form vs. oligomers derived from it.

We found that oTau does not impair the initial phase of LTP and short-term memory in *Mapt*-KO mice. This finding suggests that oTau needs endogenous tau to affect the initial phase of LTP and short-term memory. Moreover, it shows an additional parallelism between tau and Aβ, in that tau suppression

protects against the early damage of synaptic plasticity and memory caused by both oAβ and oTau.

Overexpression of human wild-type tau abolishes the protective effect tau suppression has against Aβ-induced impairment of the initial phase of LTP and short-term memory. These experiments confirm that tau is genuinely needed for these impairments to occur. Additionally, they are consistent with the idea that different memory types exist, a tau-dependent one and another one which would be tau-independent. To this end, the molecular mechanisms of the tau-independent memory could involve APP as binding between APP and tau or Aβ is required for the detrimental effects of Aβ and tau on long-term synaptic plasticity and memory (19, 27-31). Additionally, fragments of APP processing are acquiring increased attention. They could be modulated differentially in response to the various single and combined alterations of Aβ and tau. In particular, APP-βCTF is emerging as a highly relevant pathogenic factor in AD and previous work has shown similarities in the profile of synaptic and cognitive effects induced by altered levels or distributions of APP-βCTF (65-70).

The impairment of LTP and memory was present with sub-toxic doses of oAβ in htau/*Mapt*-KO mice. This is probably dependent upon the fact that these mice produce low amounts of oTau, which combined with low doses of oAβ produce full-blown impairments. Consistent with this finding, oAβ and oTau act in cooperation when they determine LTP and memory impairment (10). Moreover, dose is not the only variable in our experimental paradigms that

might have affected outcome. Other important variables that one should take into account when interpreting results are the age of the animals and the durations of the treatment. For instance, we found memory defects in *Mapt*-KO mice after the age of 10 months. For this reason, we chose to perform experiments at an age in which tau suppression does not interfere with the interpretation of our findings. Additionally, we crossed *Mapt*-KO animals with TgAPP mice with chronic expression and accumulation of naturally produced Aβ, to extend the validity of findings from experiments with acute exposure to oAβ.

A straightforward conclusion from our experiments is that anti-Aβ and anti-tau therapies alone are unlikely to effectively treat all AD symptoms. Thus, tau-targeting therapies or early intervention against Aβ are unlikely the solution to treat AD, and these data call for a reassessment of many clinical trials based on the amyloid hypothesis. Most importantly, our findings suggest that therapies targeting simultaneously Aβ and tau might effectively improve LTP and memory. This might be achieved by either combining anti-Aβ and anti-tau therapeutics, or more likely, given that the physiological functions of these proteins might render these therapeutics not clinically viable (71, 72), targeting substrates downstream of both peptides through either personalized medicine approaches or drugs acting on second messenger systems shared by the two proteins and relevant to synaptic plasticity and memory.

Methods

Animals

The following groups of mice were used: i) *Mapt*-KO and WT littermates (32, 41) (https://www.jax.org/strain/007251); ii) transgenic mice overexpressing human APP carrying the Swedish (APP KM670/671NL) and the Indiana (V717F) mutations named TgAPP mice (33) with their TgAPP*/Mapt*-KO and *Mapt*-KO littermates obtained by crossing TgAPP in a tau-hemizygous background; iii) htau*/Mapt*-KO mice obtained by crossing htau mice in a murine tau-hemyzygous background to generate htau*/Mapt*-KO mice and siblings (41). The htau animals express wild-type, full-length human tau (2N4R) driven by the prion promoter and were generated using the same approach as previously described for the R406W and P301L mutant transgenes (73, 74). Mice were obtained from breeding colonies kept in the animal facility of the University of Toronto. PCR on tail samples was used for genotyping, as previously described (73). Animals were maintained on a 12 hr light/dark cycle, in a temperature and humidity-controlled room. Food and water were available *ad libitum*. Mice were allocated to a specific treatment and paradigm by a randomization procedure. Experimenters were blind in respect to genotype and treatment. All experiments were performed on sex-balanced groups. Mice were used at 4-6 months of age, unless otherwise stated in the result section.

Electrophysiology

Electrophysiological experiments were performed as previously described (36). Briefly, following their cutting, transverse hippocampal slices (400 µm)

were transferred to a recording chamber where they were maintained at 29°C and perfused with ACSF (flow rate 2 ml/min; continuously bubbled with 95% O_2 and 5% CO_2), consisting of (in mM): NaCl (124.0), KCl (4.4), Na_2HPO_4 (1.0), $NaHCO_3$ (25.0), $CaCl_2$ (2.0), $MgCl_2$ (2.0), and glucose (10.0). Stimulation of the Schaeffer collateral fibers through a bipolar tungsten electrode permitted the recording of field extracellular recordings (fEPSP) in CA1 *stratum radiatum* with a glass pipette filled with ACSF. After evaluation of input-output relationship to measure basal synaptic transmission, a 15-min baseline was recorded every minute at an intensity eliciting a response approximately 35% of the maximum evoked response. Aβ and tau were applied for 20 min after recording of the baseline. For experiments with no application of Aβ and tau the baseline was recorded for 20 min prior to eliciting potentiation. Additionally, 1H-[1, 2, 4] oxadiazolo [4,3-a]quinoxalin-1-one (ODQ; Cayman Chemichal Company) was applied for 10 min before tetanus in a few experiments. LTP was induced through a theta-burst stimulation (4 pulses at 100 Hz, with the bursts repeated at 5 Hz and three tetani of 10-burst trains administered at 15 sec intervals). Responses were recorded for 2 hrs after tetanization and measured as fEPSP slope expressed as percentage of baseline.

Behavior

Intrahippocampal infusions of oAβ and oTau was performed following stereotaxic surgery for cannulas implantation, as previously described (19). Briefly, while anaesthetized with Avertin (500 mg/Kg), mice were implanted with a 26-gauge guide cannula into the dorsal part of the hippocampi

(coordinates from bregma: posterior = 2.46 mm, lateral = 1.50 mm to a depth of 1.30 mm). After 6–8 days of recovery, mice were bilaterally infused with oAβ or oTau or vehicle (final volume of 1 µl over 60 sec). During infusion, animals were handled gently to minimize stress. In some animals, a solution of 4% methylene blue was infused for localization of infusion cannulas after behavioral studies.

The radial arm water maze (RAWM) test was performed over 2 days as previously described (75). During the first day, mice were trained in 15 trials to identify the platform location in a goal arm by alternating between a visible and a hidden platform from trial 1 to 12. In the last four trials only a hidden platform was utilized. During the second day, the platform was hidden throughout trial 1 to 15. Errors were counted when the mice entered an arm with no platform, or failed to select an arm for 15 sec and the mouse was gently pulled back to the start arm. Each trial lasted up to 1 min. At the end of each trial, mice rested on the platform for 15 sec. The goal arm was maintained constant for all trials, with a different starting arm on successive trials. Data were analyzed and displayed as averages of blocks of 3 trials. Following RAWM testing, mice underwent a visible platform test to control for possible motivational, visual and motor defects. This consisted in a two-day test, with two sessions per day (each consisting of three 1 min trials), in which the time taken to reach a visible platform (randomly positioned in a different place each time) marked with a green flag was recorded.

Fear conditioning was performed as previously described (75, 76). Briefly, mice were handled once a day for 3 days before behavioral experiments. During the first day, mice were placed in the conditioning chamber for 2 min before the onset of a discrete tone [conditioned stimulus (CS)] (a sound that lasted 30 sec at 2800 Hz and 85 dB). In the last 2 sec of the CS, mice were given a foot shock [unconditioned stimulus (US)] of 0.80 mA for 2 sec through the bars of the floor. After the CS/US pairing, the mice were left in the conditioning chamber for 30 sec and then they were placed back in their home cages. Freezing behavior (defined as the absence of all movement except for that necessitated by breathing) was measured. The contextual fear learning was evaluated during the second day for five consecutive min. The cued fear learning was evaluated during the third day by placing the mouse in a novel context for 2 min (pre-CS test), after which they were exposed to the CS for 3 min (CS test). Sensory perception of the shock (determined 24 hrs after the cued test through threshold assessment) started with a foot shock of 0.1 mA that increased by 0.1 mA every 30 sec. We recorded the first visible, motor and vocal response.

Open Field was performed as previously described (10). Briefly, mice were left in a white arena divided into sectors (periphery and center) by black lines. Each mouse was permitted to freely explore the arena for 5 min in two consecutive days. We scored the percent time spent into the center and the number of entries into the center.

Preparation of Aβ and tau oligomers

Human $A\beta_{42}$ oligomerization was obtained as described previously (36). Briefly, a protein film was prepared by dissolving $A\beta_{42}$ lyophilized powder (Biopolymer Laboratory, UCLA, CA, USA) in 1,1,1,3,3,3-Hexafluoro-2-Propanol (HFIP) and subsequent incubation for 2 hrs at room temperature to allow complete monomerization. The Aβ film was dissolved in dimethylsulfoxide (DMSO), sonicated for 15 min, aliquoted, and stored at -20°C. To oligomerize the peptide, phosphate buffered saline (PBS) was added to an aliquot of DMSO-Aβ to obtain a 5 mM solution that was incubated for 12 hrs at 4°C. This oligomerized Aβ solution was then diluted to the final concentration in artificial cerebrospinal fluid (ACSF) consisting of: 124.0 NaCl, 4.4 KCl, 1.0 Na_2HPO_4, 25.0 $NaHCO_3$, 2.0 $CaCl_2$, 2.0 $MgCl_2$ in mM. The Aβ preparation was monitored through Western blot in which Aβ samples (prepared in non-denaturing/nonreducing conditions prior to loading) were resolved by a denaturing Tris-Tricine SDS-PAGE and probed with anti-human Aβ monoclonal antibody 6E10 (BioLegend; cat#: SIG-39320, dilution 1:1000).

Human recombinant tau 4R/2N was used to obtain tau as previously described (10, 19, 77). Oligomerization was achieved through introduction of disulfide bonds via incubation with 1 mM H_2O_2 at room temperature for 20 hrs, followed by centrifugation in PES at 4000 x g. The resulting material was used for the experiments. The tau preparation was monitored through Western blot without reducing agent, as described (77). The samples were loaded to 10% Tris-Acetate gels that transferred on nitrocellulose membrane, following a

common Western Blot protocol (anti-tau antibody RabMad EP2456Y; cat#: ab76128, dilution 1:1000).

Immunohistochemistry

For immunohistochemistry and amyloid load analyses, brain hemispheres were fixed in 10% formalin (Millipore-Sigma) overnight at 4°C then immersed in 70% ethanol. Serial sections (5 µm) of paraffin embedded tissue were stained for amyloid plaques using an Aβ-specific antibody (4G8, BioLegend; cat#: 800701, dilution 1:200), or tau using the TAU-5 antibody (ThermoFischer, Cat #MA5-12808; dilution 1:500). Plaque densities for the different groups of transgenic mice were determined as previously described (78). Briefly, immunostained sections (5 µm) were scanned with Mirax Scan (Zeiss) and assessed using ImageScope (Aperio). Slides were scanned using the Mirax Scan v. 1.11 software and Zeiss Mirax Slide Scanner at 20X magnification with a Zeiss 20X/0.8 objective lens and a Marlin F146-C CCD camera. The rendered digital images were analyzed using the Color Deconvolution Algorithm in the Aperio Imagescope software, as described previously (79). For tau immunostaining, Cy3-conjugated anti-mouse (Jackson ImmunoResearch, Cat. #115-165-146; dilution 1:1000) were used as secondary antibodies and cellular nuclei labeled with DAPI.

Western Blotting

Brain tissues were homogenized in RIPA buffer and separated on a 4-20% Tris-Glycine gradient gel (10 µg total protein/lane) and probed with a rabbit polyclonal antibody to human tau (dilution of 1:100,000; Agilent/DAKO, cat.#

A002401-2). Equal amounts of proteins were loaded into each lane. To confirm equal loading, blots were re-probed with corresponding antibody for GADPH (dilution of 1:2000; Origene, cat.#: TA8025198BM).

Total and oTau Sandwich Enzyme-linked Immunosorbent Assay (sELISA)

Brain tissue from the combined hippocampus and cortex was homogenized in lysis buffer (20mM Tris; pH 7.4, 0.25M sucrose, 1 mM EDTA, 1 mM EGTA, supplemented with protease inhibitors and PhosStop (Sigma)) using sonication. Then, 0.1% TritonX-100 was added to the samples and lysates were spun at 12,000 x g for 20 minutes at 4º C to remove cellular debris. The supernatant was collected and a protein assay was used to determine total protein content. Samples were assayed in total tau and oligomeric tau sELISAs using methods similar to those described previously (80-82). Recombinant tau proteins were generated as described previously (83). Monomeric tau and arachidonic acid-induced aggregates were produced as described (83). For total tau assays, the capture antibody was Tau12 (aa8-21, ID# AB_2721192, (80, 84) and 100 μg of lysate protein was used. For oTau assays, the oligomer-specific, TOC1 monoclonal antibody (aa209-225, ID# AB_2832939, (85, 86), was used for capture and 25 μg of lysate protein was used. The polyclonal pan-tau antibody, R1 (ID# AB_2832929) (87), was used for detection of captured tau. Monomeric tau and arachidonic acid-induced aggregates were produced as described (83). Recombinant protein standards consisting of monomeric tau (10 – 1.3 nM) or aggregated tau (0.16 – 1.3 nM) were used to estimate the level of tau present in total tau assays or oTau

assays, respectively. Absorbance was read at 450 nm using a spectrophotometer. Absorbance data from the standard curves were used to convert the brain lysate sample data to tau levels.

Statistics

Investigators who performed the experiments were blinded with respect to treatment and the genotype. Pairing between raw data and the corresponding group was performed at the end of each experimental setting. Pre-established inclusion criteria were used to select hippocampal slices for electrophysiological recordings (healthy slices with smooth edges and surface) and mice for behavioral studies (animals in general good health, averaged weight 28 ± 2 for females and 30 ± 3 for males). Animals were allocated to a specific group by a randomization procedure. Sample size was calculated by G-Power 3.1 software. Power analyses ($\alpha = 0.05$, power $1-\beta = 0.80$) suggested a minimum of 6 slices (electrophysiology) and 8 mice (behavioral studies) to obtain an effect size = 0.62.

After data collection, statistical analysis was performed by using Systat 9 software (Chicago, IL, USA; RRID:SCR_010455). A preliminary analysis of normal distribution was performed by Shapiro-Wilk normality test. For electrophysiological recordings, 1-2 slices were recorded from the same mouse and the reported N corresponds to the number of slices. Results were analyzed in pClamp 10 (Molecular Devices; RRID:SCR_011323) and compared by ANOVA with repeated measures for input/output relationship and LTP curves, one-way ANOVA with Bonferroni's post-hoc corrections for

26^{th}-30^{th} and the 116^{th}-120^{th} recording points after tetanus. For behavioral experiments, mice were distributed in a balanced fashion with respect to sex and genotype and, for each condition mice were trained and tested in three to four separate sets of experiments. Errors in the RAWM were manually counted. Freezing, latency, time spent in the center of the arena and number of entries in the center were scored by using a video-tracking recording system. We used one-way ANOVA with Bonferroni's post-hoc correction or ANOVA with repeated measures for comparisons among the groups of mice. Two-samples t test was used when comparing two conditions.

Data were expressed as mean ± standard error mean (SEM). The level of significance was set at $P<0.05$.

Study approval

All protocols involving animals complied with the ARRIVE guidelines, and were approved by the Institutional Animal Care and Use Committee at Columbia University, the University of Toronto, and the University of Catania.

AUTHOR CONTRIBUTIONS

D.P. contributed to the conceptualization, formal analysis, and investigation, as well as writing of the original draft, review, editing, and visualization of the manuscript; E.K.A. contributed to the electrophysiological experiments, and the writing of the manuscript; A.S. performed the behavioral experiments, H.Z. performed electrophysiological experiments; E.C., E.Z., E.A., and D.D.L.P. performed biochemical experiments; M.F. contributed to the

electrophysiologocal experiments and conceptualization of the manuscript; C.G. contributed to the conceptualization of the project, and the writing, review, and editing of the manuscript; L.D. contributed to the conceptualization of the project, provided resources, and participated to the writing, review and editing of the manuscript; N.M.K. and P.E.F. contributed to the conceptualization of the project and formal analysis of the data, provided resources, participated to the writing, review and editing of the manuscript, as well as funding acquisition; O.A. contributed to the conceptualization, wrote the original draft and its revision, supervised the whole work, administered the project, and acquired funds.

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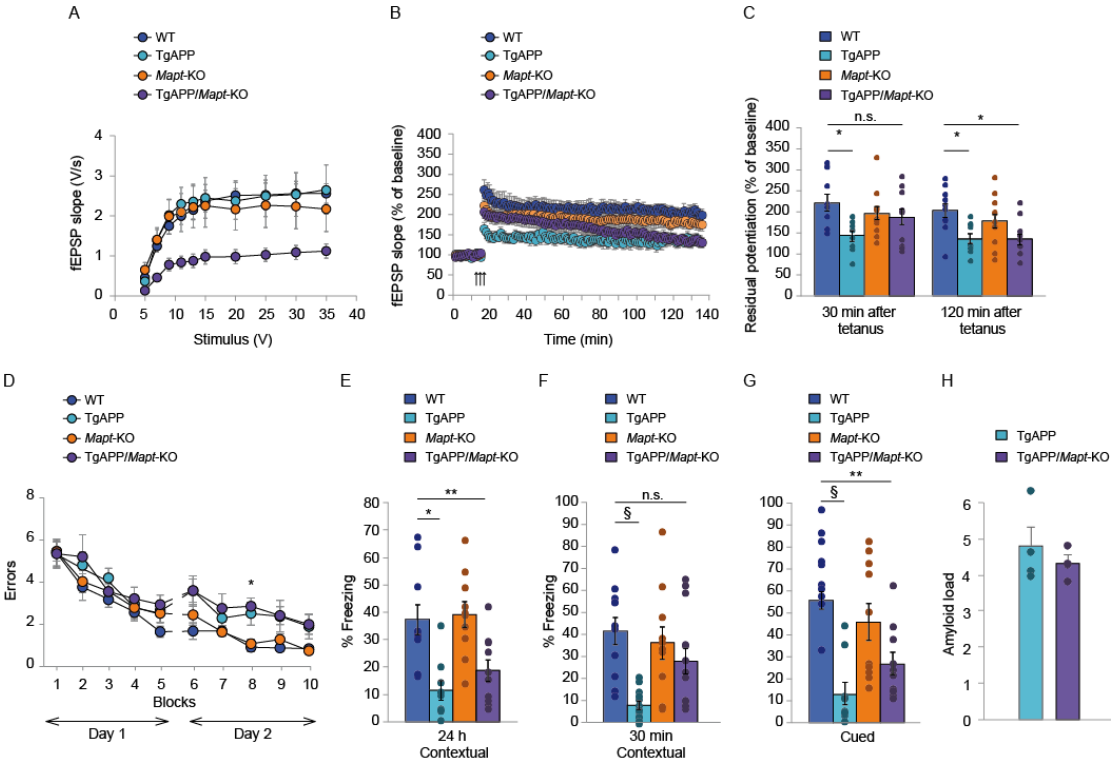

Figure 1. Mutated APP overexpression impairs long-term but not short-term synaptic plasticity and memory in *Mapt*-KO mice. (**A**) Basal neurotransmission is normal in *Mapt*-KO and TgAPP slices ($F_{(2,26)}$=1.639, *P*=0.108), but impaired in TgAPP/*Mapt*-KO slices ($F_{(1,17)}$=31.106, *P*<0.0001; *N*=9 WT, *N*=9 TgAPP, *N*=12 *Mapt*-KO, *N*=11 TgAPP/*Mapt*-KO). (**B**) Endogenous tau suppression does not protect TgAPP slices against LTP impairment ($F_{(1,18)}$=6.085, *P*=0.01, WT vs. TgAPP; $F_{(1,21)}$=5.119, *P*<0.05, WT vs. TgAPP/*Mapt*-KO; *N*=11 WT, *N*=9 TgAPP, *N*=12 *Mapt*-KO, *N*=11 TgAPP/*Mapt*-KO). (**C**) Analysis of slices displayed in (b) shows normal LTP at 30 min after tetanus in TgAPP/*Mapt*-KO slices (Bonferroni's *P*=1, WT vs. TgAPP/*Mapt*-KO), but not at 120 min (*P*<0.05). (**D**) RAWM performance is impaired in TgAPP and TgAPP/*Mapt*-KO mice (ANOVA for repeated measures, day 2: $F_{(3,39)}$=5.961, *P*=0.002; Bonferroni's *P*<0.05 in WT vs. TgAPP and *P*=0.005 vs. TgAPP/*Mapt*-KO for block 8. *N*=10 WT, *N*=11 TgAPP, *N*=10 *Mapt*-KO, *N*=12 TgAPP/*Mapt*-KO). (**E**) Contextual fear memory is impaired in TgAPP and TgAPP/*Mapt*-KO mice at 24 hrs after training (one-way ANOVA $F_{(3,35)}$=8.897, *P*<0.0001; Bonferroni's *P*<0.005, WT vs. TgAPP; *P*<0.05, WT vs. TgAPP/*Mapt*-KO mice; *N*=10 WT, *N*=9 TgAPP, *N*=10 *Mapt*-KO, *N*=10 TgAPP/*Mapt*-KO). (**F**) Endogenous tau suppression protects TgAPP mice against short-term contextual fear memory impairment (Bonferroni's *P*=0.472, WT vs. TgAPP/*Mapt*-KO mice; *N*=11 WT, *N*=13 TgAPP, *N*=10 *Mapt*-KO, *N*=13 TgAPP/*Mapt*-KO). (**G**) Cued fear memory is impaired in TgAPP and TgAPP/*Mapt*-KO mice ($F_{(3,35)}$=10.207, *P*<0.0001; Bonferroni's *P*<0.01 for both genotypes vs. WT). (**H**) Endogenous tau suppression does not influence amyloid load in TgAPP mice (*P*>0.05; *N*=4 for both groups). **P*<0.05; ***P*<0.01; §*P*<0.0001; n.s.=not significant.

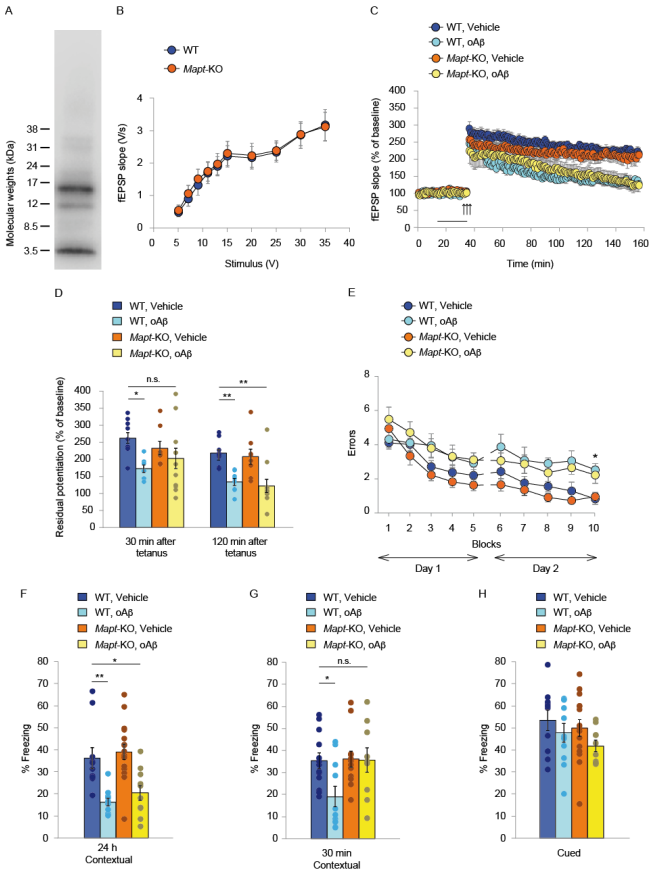

Figure 2. Extracellular oAβ impairs long-term but not short-term synaptic plasticity and memory in *Mapt-KO* mice. (**A**) Tris-Tricine SDS-PAGE Western blotting of $Aβ_{42}$ samples (prepared in non-denaturing/nonreducing conditions prior to loading) showing different bands corresponding to monomers, and oligomers. **(B)** Basal neurotransmission is similar in WT and *Mapt*-KO slices (*N*=18/17; $F_{(1,33)}$=0.031, *P*=0.861). (**C**) LTP is similar in WT and *Mapt*-KO slices (*N*=10/8; $F_{(1,16)}$=1.176, *P*=0.294). oAβ (200 nM) treatment impairs LTP in WT ($F_{(1,16)}$ = 31.192, *P*<0.0001; *N*=8) and *Mapt*-KO ($F_{(1,19)}$=6.219, *P*<0.05; *N*=8) slices. (**D**) Analysis of slices displayed in (C) shows protection against LTP impairment at 30 min after tetanus in *Mapt*-KO+oAβ slices (Bonferroni's *P*=0.282 in WT+vehicle vs. *Mapt*-KO+oAβ), but not at 120 min (*P*=0.001). (**E**) oAβ (200 nM) impairs RAWM performance in WT and *Mapt*-KO mice (day 2: $F_{(3,36)}$=5.598, *P*<0.005; Bonferroni's *P*<0.05 WT+oAβ and *Mapt*-KO+oAβ for block 10; *N*=10 WT+vehicle and *Mapt*-KO+oAβ, *N*=11 WT+oAβ, *N*=9 *Mapt*-KO+vehicle). (**F**) Contextual fear memory is impaired in WT and *Mapt*-KO mice infused with oAβ tested at 24 hrs after training ($F_{(3,42)}$=10.836, *P*<0.0001; Bonferroni's *P*<0.005 in WT+vehicle vs. WT+oAβ; *P*<0.05 in WT+vehicle vs. *Mapt*-KO+oAβ; *N*=10 WT+vehicle, *N*=11 WT+oAβ, *N*=15 *Mapt*-KO+vehicle, N=10 *Mapt*-KO+oAβ). (**G**) Endogenous tau suppression protects against oAβ-induced impairment of short-term contextual fear memory at 30 min after training ($F_{(3,41)}$=3.778, *P*<0.05; Bonferroni's *P*<0.05 in WT+vehicle vs. WT+oAβ; *P*=1 in WT+vehicle vs. *Mapt*-KO+oAβ; *N*=13 WT+vehicle, *N*=11 WT+oAβ, *N*=12 *Mapt*-KO+vehicle, *N*=9 Mapt-KO+oAβ). (**H**) No differences are detected among WT and *Mapt*-KO mice treated with vehicle or oAβ in cued conditioning test ($F_{(3,42)}$=1.347, *P*=0.272). **P*<0.05; ***P*<0.01; n.s.=not significant.

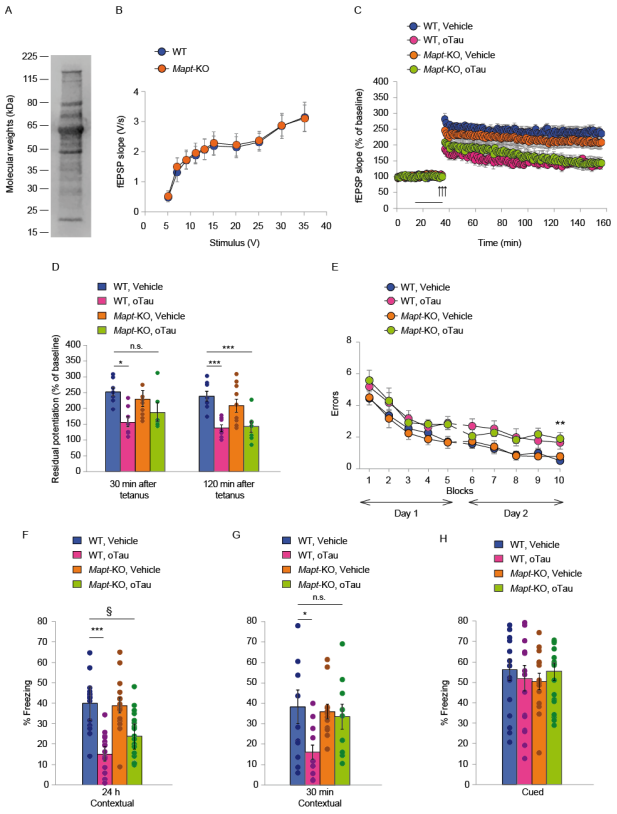

Figure 3. Extracellular oTau impairs long-term but not short-term synaptic plasticity and memory in *Mapt*-KO mice. (A) Immunoblot for recombinant tau oligomers using anti-tau antibody after isolation and oligomerization. (**B**) Basal neurotransmission is similar in WT and *Mapt*-KO slices ($F_{(1,33)}$=0.017, *P*=0.897; *N*=18/15). (**C**) oTau (50 nM) impairs LTP in WT and *Mapt*-KO slices ($F_{(1,14)}$=27.77, *P*<0.0001 in WT+vehicle vs. WT+oTau; $F_{(1,13)}$=9.44, *P*<0.01 in WT+vehicle vs. *Mapt*-KO+oTau; *N*=8 WT+vehicle, *N*=10 WT+oTau, *N*=7 *Mapt*-KO+vehicle, *N*=8 *Mapt*-KO+oTau). (**D**) Analysis of slices displayed in (C) shows protection against LTP impairment at 30 min after tetanus in *Mapt*-KO+oTau slices (Bonferroni's *P*=0.369 in WT+vehicle vs. *Mapt*-KO+oTau), but not at 120 min (*P*=0.002). (**E**) oTau (500 nM) impairs RAWM performance in WT and *Mapt*-KO mice (day 2: $F_{(3,32)}$=5.431, *P*<0.005; Bonferroni's *P*<0.05 in WT+vehicle vs. WT+oTau; *P*=0.005 in WT+vehicle vs. *Mapt*-KO+oTau for block 10; *N*=13 WT+vehicle, *N*=8 WT+oTau, *N*=8 *Mapt*-KO+vehicle, *N*=7 *Mapt*-KO+oTau). (**F**) Contextual fear memory is impaired in WT and *Mapt*-KO mice infused with oTau tested at 24 hr after training ($F_{(3,60)}$=16.541, *P*<0.0001; Bonferroni's *P*<0.0001 in WT+vehicle vs. WT+oTau; *P*=0.001 in WT+vehicle vs. *Mapt*-KO+oTau; *N*=16 WT+vehicle, *N*=16 WT+oTau, *N*=14 *Mapt*-KO+vehicle, *N*=18 *Mapt*-KO+oTau). (**G**) Endogenous tau suppression protects against the oTau-induced impairment of short-term contextual fear memory ($F_{(3,40)}$=3.463, *P*<0.05; Bonferroni's *P*<0.05 in WT+vehicle vs. WT+oTau; *P*=1 in WT+vehicle vs. *Mapt*-KO+oTau; *N*=11 WT+vehicle, *N*=12 WT+oTau, *N*=12 *Mapt*-KO+vehicle, *N*=9 *Mapt*-KO+oTau). (**H**) Cued fear memory is similar in WT and *Mapt*-KO mice treated with vehicle or oTau ($F_{(3,60)}$ = 0.269, p = 0.847). **P*< 0.05; ***P<0.005; §*P*<0.0001; n.s.=not significant.

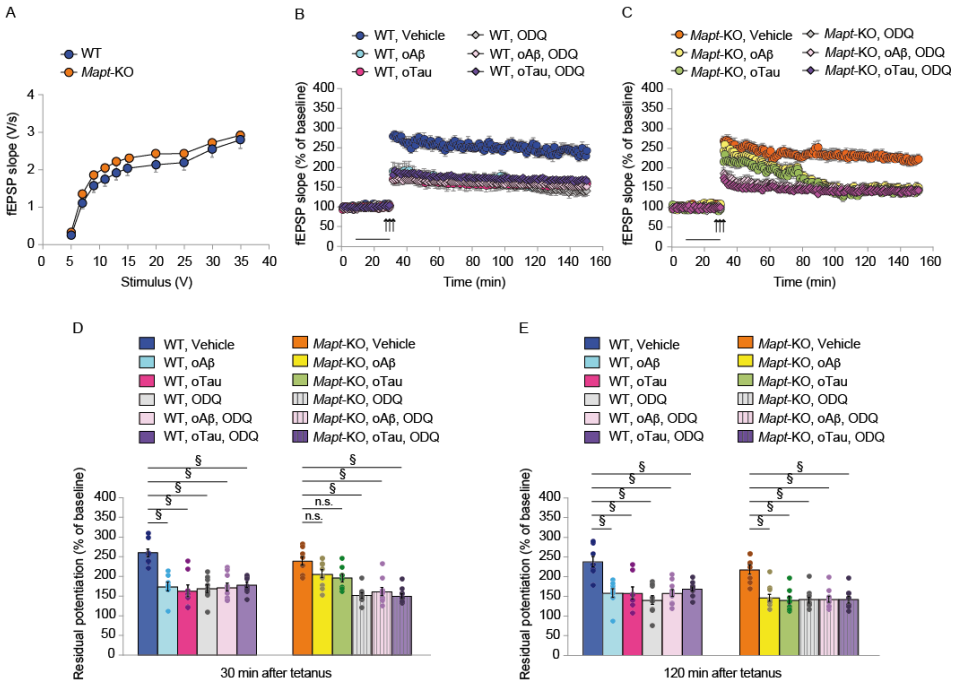

Figure 4. Inhibition of sGC abolishes the neuroprotective effect of tau suppression against oAβ- or oTau-induced impairments in short-term plasticity. (A) Basal neurotransmission is similar in WT and *Mapt*-KO slices (*N*=53/54; $F_{(1,105)}$=0.977, *P*=0.325). **(B)** Application of either oAβ (200 nM), or oTau (50 nM), or ODQ (10 µM), or oAβ+ODQ, or oTau+ODQ impairs LTP in WT slices ($F_{(1,14)}$=38.46, *P*<0.0001 in WT+vehicle vs. WT+oAβ; $F_{(1,14)}$=28.76, *P*<0.0001 in WT+vehicle vs. WT+oTau; $F_{(1,15)}$=49.97, *P*<0.0001 in WT+vehicle vs. WT+ODQ; $F_{(1,16)}$=42.90, *P*<0.0001 in WT+vehicle vs. WT+oAβ+ODQ; $F_{(1,16)}$=65.02, *P*<0.0001 in WT+vehicle vs. WT+oTau+ODQ; *N*=8 WT+vehicle, *N*=8 WT+oAβ, *N*=8 WT+oTau, *N*=9 WT+ODQ, *N*=10 WT+oAβ+ODQ, *N*=10 WT+oTau+ODQ). **(C)** Application of either oAβ or oTau or ODQ or oAβ+ODQ or oTau+ODQ impairs LTP in *Mapt*-KO slices ($F_{(1,16)}$=18.99, *P*<0.0001 in *Mapt*-KO+vehicle vs. *Mapt*-KO+oAβ; $F_{(1,14)}$=25.25, *P*<0.0001 in *Mapt*-KO+vehicle vs. *Mapt*-KO+oTau; $F_{(1,15)}$=45.32, *P*<0.0001 in *Mapt*-KO+vehicle vs. *Mapt*-KO+ODQ; $F_{(1,16)}$=40.90, *P*<0.0001 in *Mapt*-KO+vehicle vs. *Mapt*-KO+oAβ+ODQ; $F_{(1,15)}$=46.40, *P*<0.0001 in *Mapt*-KO+vehicle vs. *Mapt*-KO+oTau+ODQ; *N*=8 *Mapt*-KO+vehicle, *N*=10 *Mapt*-KO+oAβ, *N*=8 *Mapt*-KO+oTau, *N*=9 *Mapt*-KO+ODQ, *N*=10 *Mapt*-KO+oAβ+ODQ, *N*=9 *Mapt*-KO+oTau+ODQ). These experiments were interleaved with those displayed in B. (**D**) Analysis of slices displayed in (B and C) shows LTP impairment in WT slices treated with oAβ or oTau (Bonferroni's *P*<0.0001 vehicle vs. oAβ or oTau), but not in *Mapt*-KO slices treated with oAβ or oTau (*P*>0.05 vehicle vs. oAβ/oTau) at 30 min after the tetanus. ODQ perfusion unraveled LTP defect in oAβ- or oTau-treated *Mapt*-KO slices (*P<0.05* vehicle vs. ODQ+ oAβ/oTau) at 30 min after the tetanus. ODQ did not further depress LTP in WT slices treated with oAβ- or oTau (*P* = 1 ODQ vs. oAβ/oTau+ODQ) at 30 min after tetanus. (**E**) The same slices as in D showed LTP impairment at 120 min after the tetanus regardless of the treatment with oAβ/oTau/ODQ/ODQ+oAβ/oTau both in WT (Bonferroni's *P*<0.0001) and *Mapt*-KO slices (*P*<0.0001) at 120 min after tetanus. §*P*< 0.0001; n.s.=not significant.

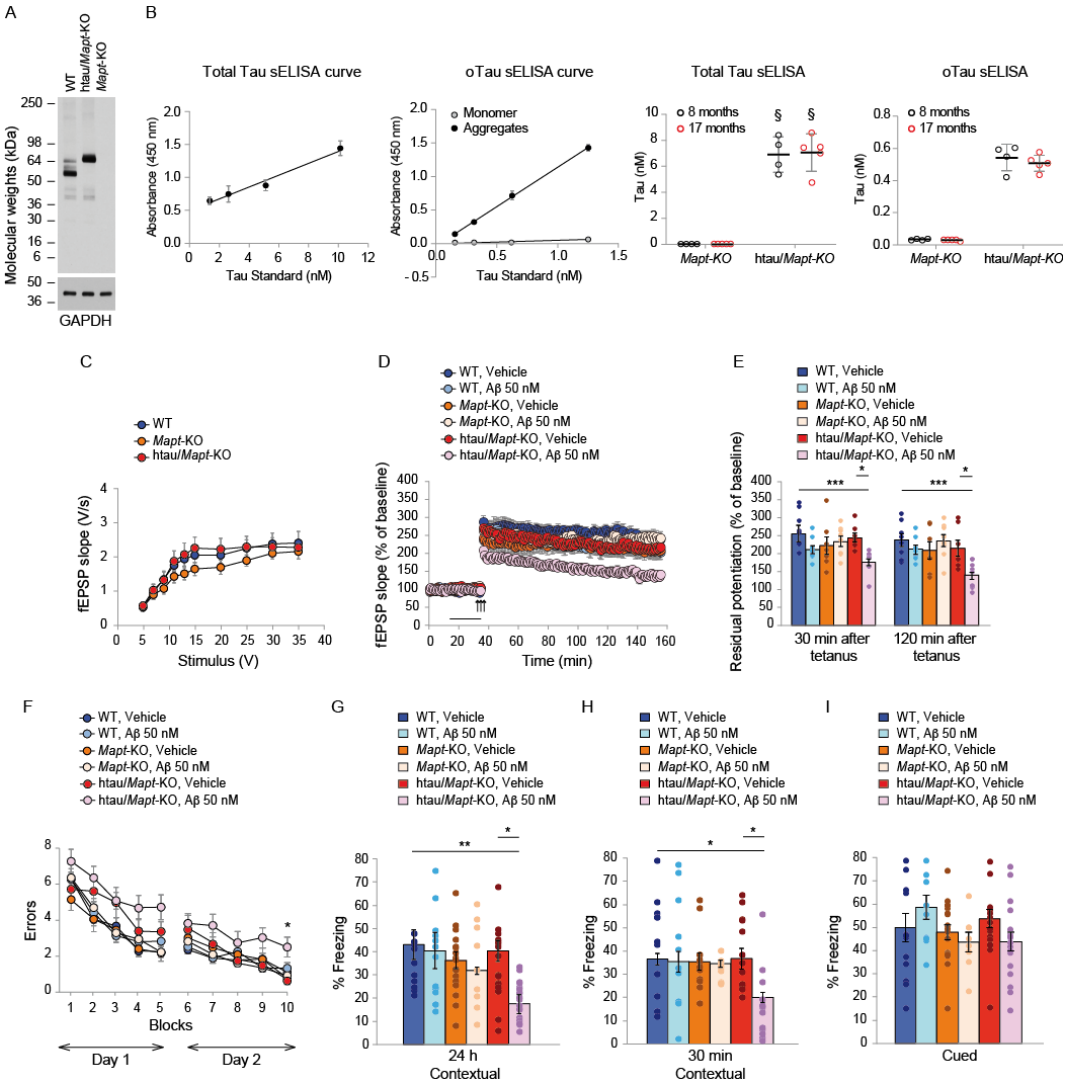

Figure 5. Reconstitution of Tau expression re-establishes oAβ-induced disruption of short-term synaptic plasticity and memory. (**A**) Similar levels of human and murine tau expression in htau/*Mapt*-KO and WT mice. (**B**) Tau monomer (1.3 – 10 nM) standard curve (r^2 = 0.92) used to interpolate total tau and tau aggregate (0.16 – 1.3 nM) standard curve (r^2 = 0.99) used to interpolate oTau. Assessment of total tau in hippocampus/cortex of 8- and 17-month-old htau/*Mapt*-KO mice (*P*<0.0001 compared to *Mapt*-KO at 8 months, *N*=4/4 and 17 months, *N*=5/5), and oTau levels (*P*<0.0001 compared to *Mapt*-KO at 8 months, *N*=4/4 and 17 months, *N*=5/5). Note the lack of signal in tau monomer standard curve in oTau assays demonstrating the specificity for oligomeric species. (**C**) Basal neurotransmission is similar in WT, *Mapt*-KO and htau/*Mapt*-KO slices ($F_{(2,40)}$=3.865, *P*=0.639; *N*=15/11/17, respectively). (**D**) Subtoxic extracellular oAβ (50 nM) impairs LTP in htau/*Mapt*-KO slices ($F_{(1,15)}$=33.474, *P*<0.0001 vs. WT+vehicle; *N*=8 WT+vehicle, *N*=8 WT+oAβ, *N*=7 *Mapt*-KO+vehicle, *N*=8 *Mapt*-KO+oAβ, *N*=7 htau/*Mapt*-KO+vehicle, *N*=9 htau/*Mapt*-KO+oAβ). (**E**) Analysis of slices displayed in (d) shows LTP impairment at 120 and 30 min after tetanus (Bonferroni's *P*<0.05 vs. WT or htau/*Mapt*-KO+vehicle). (**F**) Subtoxic oAβ (75

nM) impairs RAWM performance in htau/*Mapt*-KO mice (day 2 $F_{(5,73)}$=3.412, *P*=0.008; Bonferroni's *P*<0.05 vs. WT+vehicle; *P*<0.005 vs. htau/*Mapt*-KO+vehicle, block 10; *N*=13 WT+vehicle, *N*=13 WT+oAβ, *N*=12 *Mapt*-KO+vehicle, *N*=14 *Mapt*-KO+oAβ, *N*=14 htau/*Mapt*-KO+vehicle, *N*=14 htau/*Mapt*-KO+oAβ). (**G-H**) oAβ 75 nM impairs contextual fear memory in htau/*Mapt*-KO mice at 24 hrs ($F_{(5,86)}$=3.504, *P*<0.01, Bonferroni's *P*<0.05; *N*=13 WT+vehicle, *N*=16 WT+oAβ, *N*=18 *Mapt*-KO+vehicle, *N*=9 *Mapt*-KO+oAβ, *N*=19 htau/*Mapt*-KO+vehicle, *N*=18 htau/*Mapt*-KO+oAβ) and 30 min after training ($F_{(5,62)}$=3.897, P<0.005; Bonferroni's *P*<0.05, *N*=11 WT+vehicle, *N*=10 WT+oAβ, *N*=12 *Mapt*-KO+vehicle, *N*=10 *Mapt*-KO+oAβ, *N*=12 htau/*Mapt*-KO+vehicle, N=13 htau/*Mapt*-KO+oAβ). (**I**) Cued fear memory is similar in the six groups of mice displayed in (G) ($F_{(5,79)}$=1.481, *P*=0.205). **P*<0.05; ***P*<0.01; ****P*<0.005; n.s.=not significant.

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