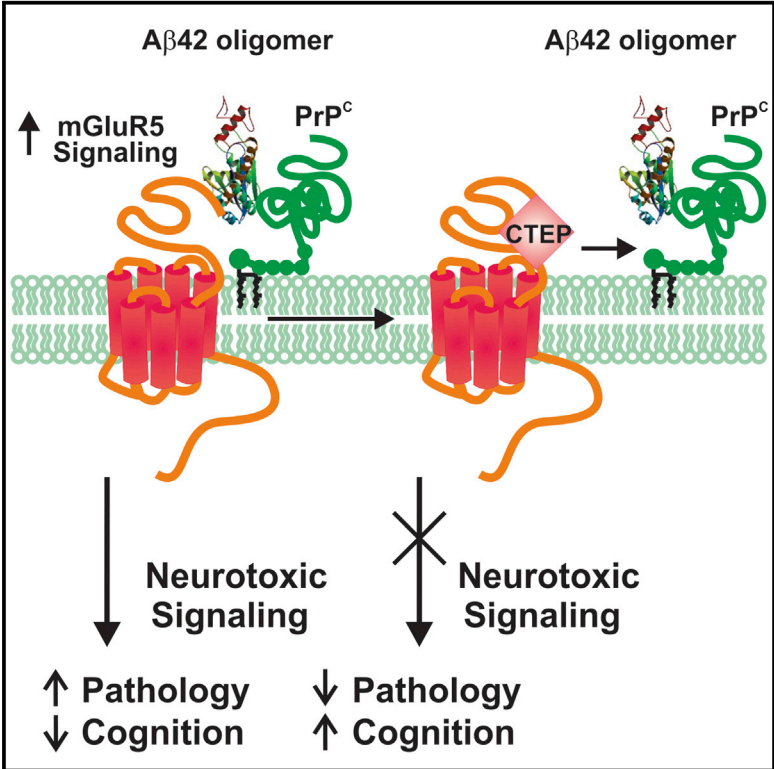


Chronic Pharmacological mGluR5 Inhibition Prevents Cognitive Impairment and Reduces Pathogenesis in an Alzheimer Disease Mouse Model

Graphical Abstract



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In Brief

Hamilton et al. demonstrate that chronic treatment of an Alzheimer disease mouse model with Basimglurant (CTEP) rescues the cognitive function and reduces disease pathology. The chronic pharmacological inhibition of mGluR5 signaling with CTEP might be effective for the treatment of cognitive deficits experienced by AD patients.

Highlights

- Chronic treatment with CTEP improves memory and cognitive function in an AD mouse
- Chronic treatment with CTEP reduces Aβ oligomers and Aβ plaques in an AD mouse
- mGluR5 antagonism may be a viable approach for the treatment of AD



Chronic Pharmacological mGluR5 Inhibition Prevents Cognitive Impairment and Reduces Pathogenesis in an Alzheimer Disease Mouse Model

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SUMMARY

Beta-amyloid (A β) oligomers contribute to the pathophysiology of Alzheimer disease (AD), and metabotropic glutamate receptor 5 (mGluR5) has been shown to act as a receptor for both A β oligomers and cellular prion proteins. Furthermore, the genetic deletion of mGluR5 in an APP^{swe}/PS1 Δ E9 mouse model of AD improves cognitive function and reduces A β plaques and A β oligomer concentrations. Here, we show that chronic administration of the orally bioavailable mGluR5-selective negative allosteric modulator CTEP, which is similar in structure, potency, and selectivity to Basimglurant (RO4917523), which is currently in phase II clinical development for major depressive disorder and fragile X syndrome, reverses cognitive decline in APP^{swe}/PS1 Δ E9 mice and reduces A β plaque deposition and soluble A β oligomer concentrations in both APP^{swe}/PS1 Δ E9 and 3xTg-AD male mice. These findings suggest that CTEP or its analogue Basimglurant might potentially be an effective therapeutic for the treatment of AD patients.

INTRODUCTION

Alzheimer disease (AD) is the most prevalent neurodegenerative disease, and the predominant neurotoxic species in the brains of AD patients is beta-amyloid (A β) protein, which is formed by the sequential cleavage of amyloid precursor protein (APP) (Citron et al., 1996; Kamenetz et al., 2003). These oligomers have been shown to be the most harmfully correlated with AD pathogenesis (McGowan et al., 2005; Shankar et al., 2008). Metabotropic glutamate receptor 5 (mGluR5) is a member of the G protein-coupled receptor (GPCR) superfamily that is activated by glutamate to couple to the heterotrimeric G protein G $\alpha_{q/11}$, resulting in the downstream second messenger-dependent release of intracellular Ca²⁺ that has been linked to a number of neurodegenerative

diseases (Ribeiro et al., 2010). A β oligomers and cellular prion protein (PrP^C) interact with mGluR5 to cause the release of Ca²⁺ from intracellular stores, thus disrupting normal neuronal signaling and function (Renner et al., 2010; Sokol et al., 2011; Um et al., 2013; Haas et al., 2014). The genetic deletion of mGluR5 improves AD pathogenesis and cognitive decline in the APP^{swe}/PS1 Δ E9 mouse model of AD (Hamilton et al., 2014). Preclinical trials in fragile X syndrome and major depressive disorder with 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl) ethynyl) pyridine (CTEP) suggest that this mGluR5-selective allosteric modulator may be effective for the treatment of AD (Lindemann et al., 2011, 2015; Michalon et al., 2012). CTEP has high in vivo potency and is an orally bioavailable, brain-permeant, mGluR5-negative allosteric modulator of mGluR5; it has a half-life of 18 hr in vivo, allowing once a day administration that is effective for the treatment of fragile X in adult mice (Lindemann et al., 2011; Michalon et al., 2012). Moreover, CTEP is highly selective for mGluR5 versus more than 100 GPCR targets tested (Lindemann et al., 2011).

In the present study, we tested whether acute and/or chronic treatment with CTEP improved cognitive performance and reduced A β pathology in APP^{swe}/PS1 Δ E9 male mice. We found that chronic, but not acute, administration of CTEP by intraperitoneal injection rescues memory deficits in novel object recognition, Morris water maze (MWM), and reversal MWM (RMWM) memory paradigms in APP^{swe}/PS1 Δ E9 mice. Chronic CTEP treatment also significantly reduced A β oligomer concentrations and plaque formation in both APP^{swe}/PS1 Δ E9 and 3xTg-AD mouse models of AD. Our data provide further evidence of a central role for mGluR5 in AD, and they highlight the potential for repurposing the CTEP analogue Basimglurant as a treatment for AD.

RESULTS

Chronic, but Not Acute, Treatment with CTEP Rescues Memory Loss

To test whether CTEP treatment would ameliorate the behavioral and pathological phenotypes observed in AD mouse models, wild-type C57Bl/6, APP^{swe}/PS1 Δ E9, and 3xTg-AD mice male mice were raised to 9 months of age and treated with either

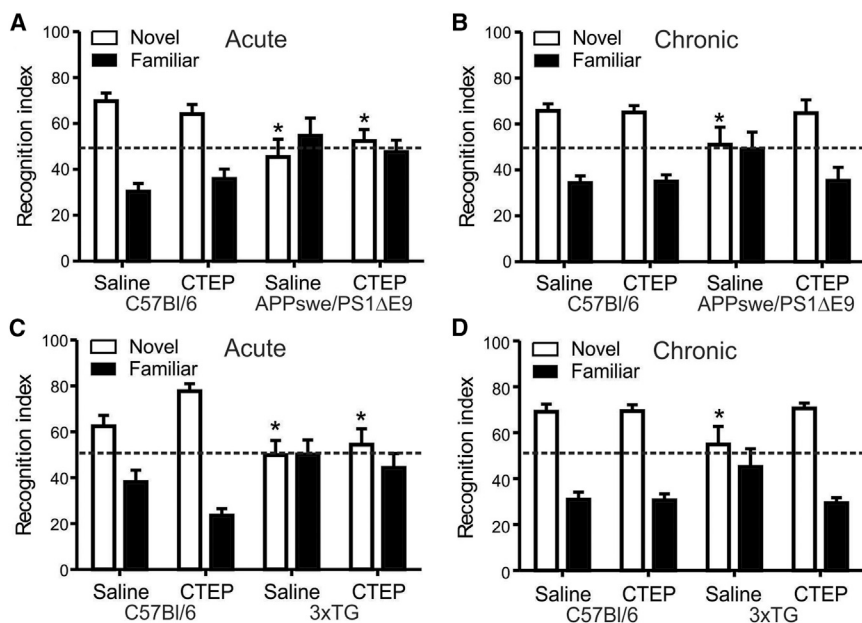


Figure 1. Chronic, but Not Acute, Administration of CTEP Rescues Recognition Memory in APPswe/PS1ΔE9 and 3xTg-AD Mice

(A) The recognition index is shown for acute (5-day) drug administration, when mice explore two identical objects and one novel versus familiar object in saline-treated C57Bl/6 mice ($n = 11$), CTEP-treated C57Bl/6 mice (2 mg/kg; $n = 12$), saline-treated APPswe/PS1ΔE9 mice ($n = 6$), and CTEP-treated APPswe/PS1ΔE9 mice (2 mg/kg; $n = 10$).

(B) The same as (A) is shown again following chronic (10- to 12-week) drug treatment in saline-treated C57Bl/6 mice ($n = 10$), CTEP-treated C57Bl/6 mice ($n = 12$), saline-treated APPswe/PS1ΔE9 mice ($n = 7$), and CTEP-treated APPswe/PS1ΔE9 mice ($n = 10$).

(C) The recognition index is shown for acute (5-day) drug administration, when mice explore two identical objects and one novel versus familiar object in saline-treated C57Bl/6 mice ($n = 11$), CTEP-treated C57Bl/6 mice (2 mg/kg; $n = 11$), saline-treated 3xTg-AD mice ($n = 7$), and CTEP-treated 3xTg-AD mice (2 mg/kg; $n = 10$).

(D) The same as (C) is shown again following chronic (10- to 12-week) drug treatment in saline-

treated C57Bl/6 mice ($n = 12$), CTEP-treated C57Bl/6 mice ($n = 12$), saline-treated 3xTg-AD mice ($n = 8$), and CTEP-treated 3xTg-AD mice ($n = 9$). Data represent the mean \pm SEM. Statistical significance is assessed by two-way ANOVA followed by Newman-Keuls multiple comparisons post hoc test ($p < 0.05$ versus saline-treated mice).

saline or CTEP (2 mg/kg) by intraperitoneal injection every 48 hr for a duration of 3 months. This drug dose was chosen based on the effective CTEP concentration utilized to treat fragile X mice (Michalon et al., 2012). Mice were first tested for the acute effects of drug treatment on cognitive behavior 5 days following the initiation of drug treatment using a novel object recognition test for recognition/episodic memory. The ANOVA for the recognition index (presence in novel area) indicated that there were differences for APPswe/PS1ΔE9 versus C57Bl/6 mouse types ($F [1, 34] = 13.00$; $p = 0.001$) (Figure 1A). In contrast, analysis of the recognition scores for chronically saline- and CTEP-treated C57Bl/6 and APPswe/PS1ΔE9 mice revealed no significant differences between groups ($F [1, 37] = 2.55$; $p = 0.12$) (Figure 1B). However, as a clear a priori hypothesis had been made, follow-up statistical testing revealed that chronically CTEP-treated APPswe/PS1ΔE9 mice exhibited recognition scores that were significantly higher than those of saline-treated APPswe/PS1ΔE9 mice. Similarly, there were significant differences in the recognition indexes for C57Bl/6 and 3xTg-AD mice that were acutely treated with and without CTEP ($F [1, 28] = 10.56$; $p = 0.003$) (Figure 1C). Chronic saline CTEP treatment of C57Bl/6 and 3xTg-AD mice did not result in significant differences between groups ($F [1, 33] = 2.52$; $p = 0.12$), but the recognition scores for chronically CTEP 3xTg-AD mice were significantly higher than those of saline-treated 3xTg-AD mice (Figure 1D).

Wild-type (C57Bl/6) and APPswe/PS1ΔE9 mice subsequently were tested in the MWM on days 6–10, which involved exposure to the maze for 16 90-s trials over 4 days during the acquisition phase (four trials per day), followed by a 60-s probe trial on day 5. Then 1 day after the completion of the MWM,

RMWM training commenced using the same protocol, except that the location of the platform was moved. For both tests we assessed escape latency, path length, and swim speed. In the MWM, latency to find the platform and the path length largely paralleled each other, and, in both cases, performance varied as a function of the strain \times drug \times trial treatment ($F[3, 72] = 10.95$ and 11.67 ; p values < 0.001 , respectively, for acutely saline- and CTEP-treated wild-type and APPswe/PS1ΔE9 mice) (Figures 2A and 2B). The follow-up tests indicated, as shown in Figures 2A and 2B, that in wild-type mice the CTEP treatment modestly reduced response latencies and path length on the initial day of testing. In saline-treated APPswe/PS1ΔE9 mice, performance on these measures did not differ from that of similarly treated wild-type mice. However, among the APPswe/PS1ΔE9 mice, the CTEP treatment significantly increased response latencies and path length relative to both the saline-treated APPswe/PS1ΔE9 mice and the wild-type mice treated with CTEP. The swim speed of mice declined over days ($F[3, 105] = 11.24$; $p < 0.001$) and varied as a function of the drug treatment \times days interaction ($F[3, 105] = 4.65$; $p < 0.01$). The follow-up tests indicated that swim speed in CTEP mice was somewhat faster than in saline-treated animals, although the magnitude of this effect was small (Figure 2C). In essence, the CTEP treatment modestly enhanced initial performance in wild-type mice, but it disrupted performance in the APPswe/PS1ΔE9 mice. There were no observed differences between mouse groups ($F[3, 43] = 1.59$; $p = 0.21$) for the time spent in the target quadrant of the maze following the removal of the platform (Figure 2D).

The analyses of MWM performance resulting from chronic CTEP treatment revealed outcomes that were distinct from

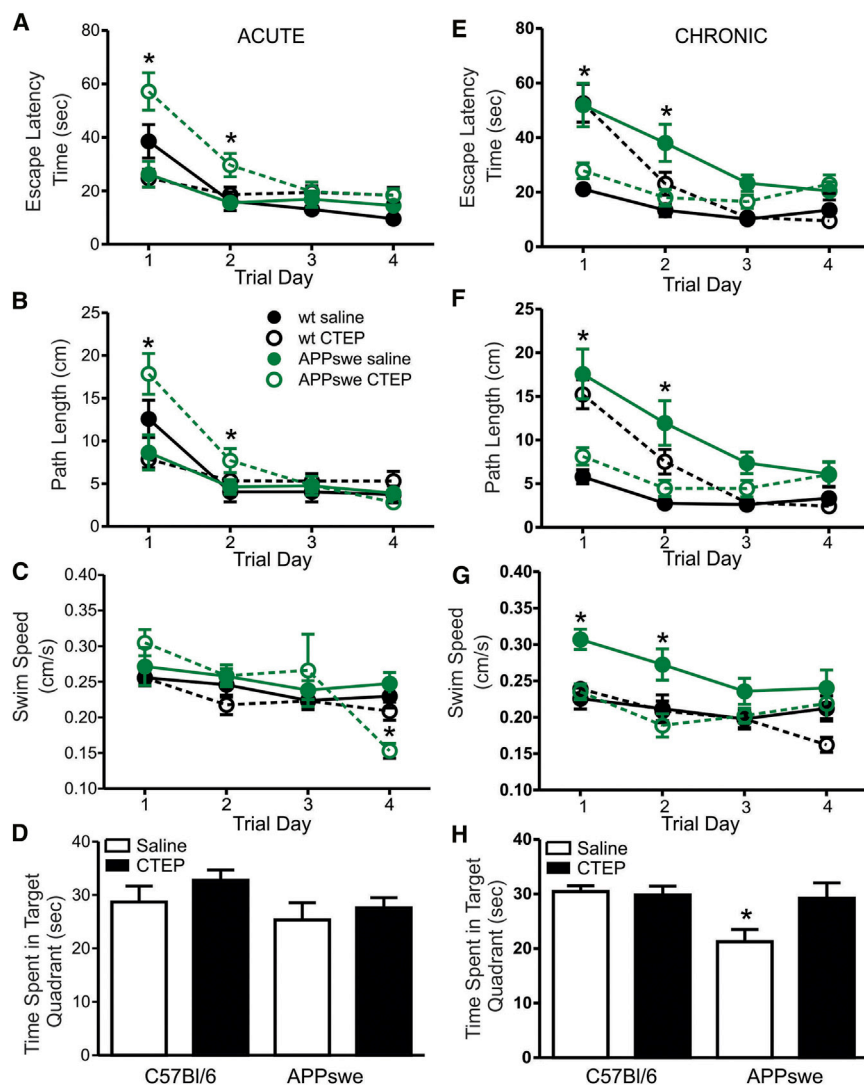


Figure 2. Performance of APPswe/PS1ΔE9 Mice in the MWM following Either Acute or Chronic Treatment with CTEP

(A–D) Shown are the results obtained for MWM acquisition phase for (A) escape latency, (B) distance traveled, (C) swim speed, and (D) time spent in the target quadrant during the probe trial for C57BI/6 and APPswe/PS1ΔE9 mice acutely treated with either saline or CTEP.

(E–H) Shown are the results obtained for MWM acquisition phase for (E) escape latency, (F) distance traveled, (G) swim speed, and (H) time spent in the target quadrant during the probe trial for C57BI/6 and APPswe/PS1ΔE9 mice chronically treated with either saline or CTEP for 10 weeks. Data are shown for saline-treated C57BI/6 (wild-type [WT]) mice ($n = 12$), CTEP-treated C57BI/6 (WT) mice ($n = 13$), saline-treated APPswe/PS1ΔE9 (APPswe) mice ($n = 9$), and CTEP-treated APPswe/PS1ΔE9 (APPswe) mice ($n = 12$). Data represent the mean \pm SEM. Statistical significance is considered $p < 0.05$, represented by * $p < 0.05$ and ** $p < 0.01$; differences are between mice groups when compared to saline-treated wild-type mice. Statistical significance is assessed by two-way ANOVA followed by Newman-Keuls multiple comparisons post hoc test (* $p < 0.05$).

the removal of the platform. Saline-treated APPswe/PS1ΔE9 mice spent the least time in the target quadrant when compared to the other mouse groups (Figure 3H). Taken together, these data indicated that long-term CTEP treatment prevents the development of spatial memory impairment in APPswe/PS1ΔE9 mice.

For the RMWM, among mice that received acute CTEP treatment the escape latencies, path length, and swim

speed during the reversal did not vary as a function of the strain, drug, or trial for any interactions involving these variables (Figures 3A–3C). There also were no observed differences between mouse groups ($F[3, 42] = 1.103$; $p = 0.36$) for the time spent in the target quadrant of the maze following the removal of the platform (Figure 3D). However, a very different picture emerged in response to the chronic treatment condition. In this instance, latencies and path length varied with the strain \times CTEP \times Trial interaction ($F[3, 126] = 7.07$ and 10.7 ; $p < 0.001$) (Figures 3E and 3F), but swim speed did not vary with the treatments (Figure 3G). Curiously, APPswe/PS1ΔE9 mice chronically treated with saline exhibited a marked deterioration of performance over days. This was obviously very different from the performance seen in acutely treated mice, although it's not certain whether this was a result of mice being 3 months older or the stress experienced with repeated injections. Similar to what was observed in the MWM, saline-treated APPswe/PS1ΔE9 mice spent the least time in the target quadrant when compared to the other mouse groups ($F[3, 42] = 7.55$; $p < 0.001$) (Figure 3H).

the effects attributable to acute CTEP administration (Figures 2F–2H). In this instance, the ANOVA revealed that escape latencies, path length, and swim speed varied as a function of the strain \times CTEP \times trial treatment interaction ($F[3, 95] = 17.93$, 18.69 , and 3.99 ; p values < 0.001 , respectively) (Figures 2E–2G). The follow-up comparisons of the simple effects of significant interactions, depicted in Figures 3E and 3F, indicated that for wild-type mice the CTEP treatment disrupted escape latencies and increased path length on the initial 2 days of testing relative to saline-treated mice. The APPswe/PS1ΔE9 mice treated with saline exhibited significantly longer escape latencies and greater path lengths than did similarly treated wild-type mice. However, unlike the effects seen in the wild-type mice, the CTEP treatment provoked markedly shorter escape latency and shorter path lengths than did APPswe/PS1ΔE9 mice treated with saline or wild-type mice that received CTEP. Moreover, significant differences were observed between mouse groups ($F[3, 41] = 3.243$; $p < 0.05$) for the time spent in the target quadrant of the maze following

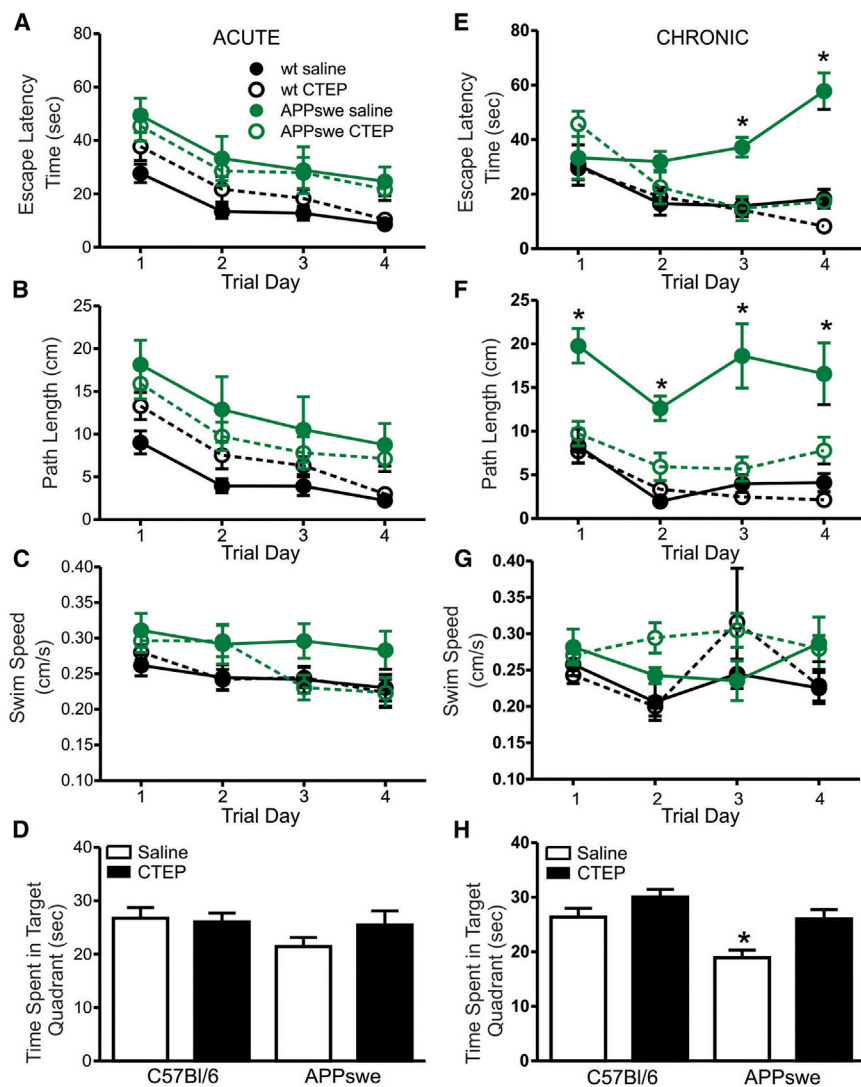


Figure 3. Performance of APPsw/PS1ΔE9 Mice in the RMWM following Either Acute or Chronic Treatment with CTEP

(A–D) Shown are the results obtained for RMWM acquisition phase for (A) escape latency, (B) distance traveled, (C) swim speed, and (D) time spent in the target quadrant during the probe trial for C57Bl/6 and APPsw/PS1ΔE9 mice acutely treated with either saline or CTEP.

(E–H) Shown are the results obtained for RMWM acquisition phase for (E) escape latency, (F) distance traveled, (G) swim speed, and (H) time spent in the target quadrant during the probe trial for C57Bl/6 and APPsw/PS1ΔE9 mice chronically treated with either saline or CTEP for 10 weeks. Data are shown for saline-treated C57Bl/6 (WT) mice (n = 12), CTEP-treated C57Bl/6 (WT) mice (n = 13), saline-treated APPsw/PS1ΔE9 (APPsw) mice (n = 9), and CTEP-treated APPsw/PS1ΔE9 (APPsw) mice (n = 12). Data represent the mean ± SEM. Statistical significance is considered $p < 0.05$, represented by * $p < 0.05$; differences are between mice groups when compared to saline-treated wild-type mice. Statistical significance is assessed by two-way ANOVA followed by Newman-Keuls multiple comparisons post hoc test (* $p < 0.05$).

with CTEP when compared to mice treated with saline (Figure 4D).

To assess the effect of chronic CTEP treatment on A β oligomer load in APPsw/PS1ΔE9 and 3xTg-AD mice, whole brains from saline- and CTEP-treated mice were analyzed for A β oligomers using a sandwich ELISA assay. We found a statistically significant difference in A β oligomer concentrations in saline- and CTEP-treated wild-type and APPsw/PS1ΔE9 mice ($F[3, 16] = 46.09$;

Taken together, these data indicated that long-term CTEP treatment prevents the development of spatial memory impairment in APPsw/PS1ΔE9 mice.

Chronic Treatment with CTEP Reduces A β

The accumulation of A β in APPsw/PS1ΔE9 mice occurs as early as 3 months of age (Lalonde et al., 2005). Therefore, to determine whether the improved cognitive performance of the APPsw/PS1ΔE9 and 3xTg-AD mice was associated with a reduction in AD pathology, we assessed whether A β plaques and A β oligomers were reduced in CTEP-treated APPsw/PS1ΔE9 and 3xTg-AD mice (Figures 4A and 4B). We found that A β plaque number was reduced in coronal cortical brain slices derived from both APPsw/PS1ΔE9 ($p < 0.0001$) and 3xTg-AD ($p < 0.001$) mice treated for 3 months with CTEP when compared to mice treated with saline (Figure 4C). Similarly, A β plaque number was reduced in coronal hippocampal brain slices derived from both APPsw/PS1ΔE9 ($p < 0.001$) and 3xTg-AD ($p = 0.001$) mice treated for 3 months

$p < 0.001$) (Figure 4E). A β oligomer concentrations were 4.0 ± 0.5 -fold higher in saline-treated APPsw/PS1ΔE9 ($p < 0.001$) mice compared to saline-treated wild-type controls, and CTEP treatment significantly reduced A β oligomer concentrations in APPsw/PS1ΔE9 mice ($p < 0.001$). However, A β oligomer concentrations were higher in both wild-type and APPsw/PS1ΔE9 mice treated with CTEP compared to saline-treated wild-type mice ($p < 0.05$). Similarly, we found a statistically significant difference in A β oligomer concentrations in saline- and CTEP-treated wild-type and 3xTg-AD mice ($F[3, 16] = 10.92$; $p = 0.001$) (Figure 4F). A β oligomer concentrations were 2.3 ± 0.5 -fold higher in saline-treated 3xTg-AD ($p < 0.001$) mice compared to saline-treated wild-type controls, and CTEP treatment significantly reduced A β oligomer concentrations in 3xTg-AD mice ($p < 0.01$). However, A β oligomer concentrations were no different in wild-type and 3xTg-AD mice treated with CTEP when compared to saline-treated wild-type mice. Thus, pharmacological blockade of mGluR5 not only improves the cognitive performance of APPsw/PS1ΔE9 mice but also it

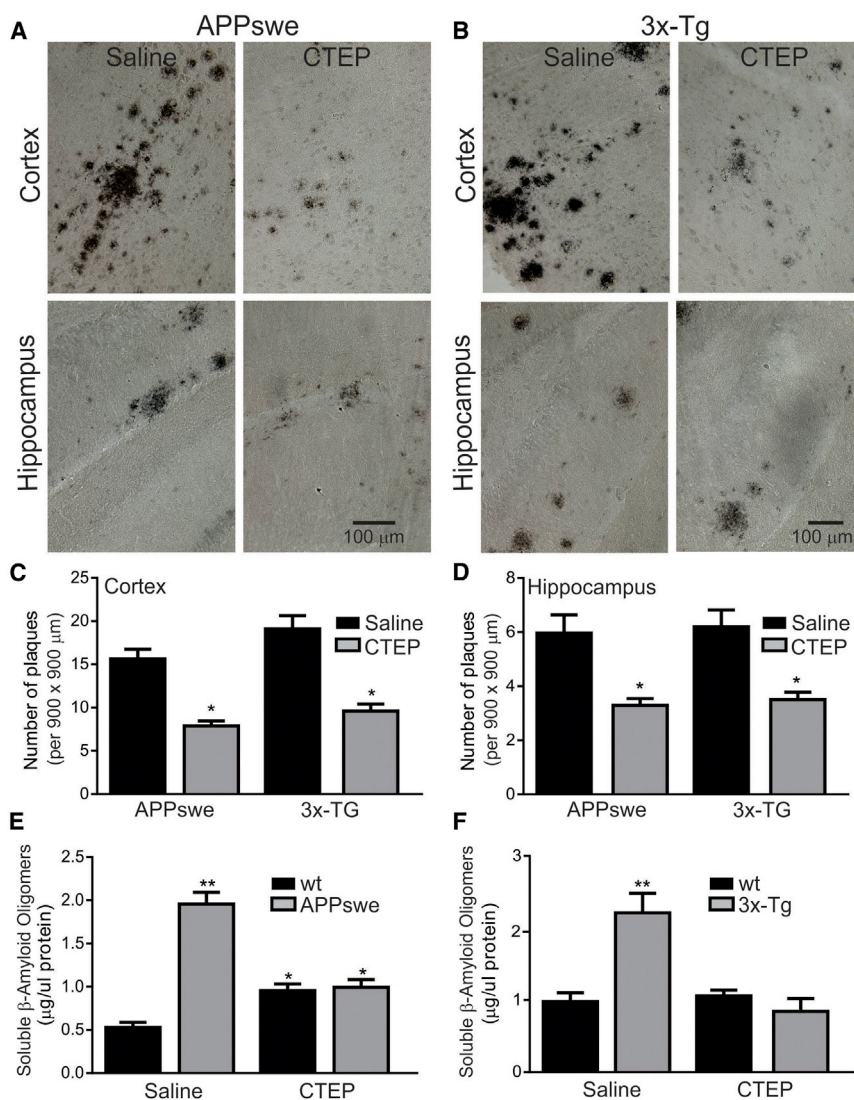


Figure 4. A β Formation and Plaque Number Are Reduced in APPswe/PS1 Δ E9 and 3xTg-AD Mice following Chronic Treatment with CTEP

(A) Representative images of A β plaques from the cortex and hippocampus of APPswe/PS1 Δ E9 mice treated with either saline or CTEP for 3 months are shown. Scale bar, 100 μ m.

(B) Representative images of A β plaques from the cortex and hippocampus of 3xTg-AD mice treated with either saline or CTEP for 3 months are shown. Scale bar, 100 μ m.

(C) The graph shows the number of plaques counted in 900 \times 900- μ m regions of interest in the cortex in coronal brain slices from APPswe/PS1 Δ E9 and 3xTg-AD mice chronically treated with either saline or CTEP. Data represent the mean \pm SEM of four to six independent experiments. Statistical significance is assessed by unpaired t test.

(D) The graph shows the number of plaques counted in 900 \times 900- μ m regions of interest in the hippocampus in coronal brain slices from APPswe/PS1 Δ E9 and 3xTg-AD mice chronically treated with either saline or CTEP. Data represent the mean \pm SEM of four to six independent experiments. Statistical significance is assessed by unpaired t test.

(E) The whole-brain A β oligomer (ng/mg protein) concentrations in 12-month-old C57Bl/6 (WT) and APPswe/PS1 Δ E9 (APPswe) mice chronically treated with either saline or CTEP. Data represent the mean \pm SEM of four independent experiments. Statistical significance is assessed by one-way ANOVA and Tukey's multiple comparison post hoc test (* p < 0.001).

(F) The whole-brain A β oligomer (ng/mg protein) concentrations in 12-month-old C57Bl/6 (WT) and 3xTg-AD mice chronically treated with either saline or CTEP. Data represent the mean \pm SEM of four independent experiments. Statistical significance is assessed by one-way ANOVA and Tukey's multiple comparison post hoc test (** p < 0.001 and * p < 0.05 versus saline control).

reduces AD-like neuropathology in two different AD mouse models.

DISCUSSION

AD is the most common cause of dementia in adults and involves a loss of memory and other cognitive abilities as the consequence of neurodegeneration, mediated in part by both soluble A β oligomers and neurofibrillary tangles that alter synaptic signaling by promoting synaptic loss (Lesné et al., 2006). We previously reported that genetic deletion of mGluR5 in the APPswe/PS1 Δ E9 mouse model of AD results in the amelioration of cognitive decline and reduces A β plaque deposition and soluble A β oligomer concentrations (Hamilton et al., 2014). In this study, we provide evidence that the selective blockade of mGluR5 activity with the high-affinity, orally bioavailable, negative allosteric modular CTEP also improves cognitive behavioral performance in an APPswe/PS1 Δ E9 AD mouse model. This is paralleled by

a CTEP-dependent reduction in the development of AD-like neuropathology in both APPswe/PS1 Δ E9 and 3xTg-AD mouse cortical and hippocampal brain regions.

This study extends our previous work demonstrating that the genetic knockout of mGluR5 corrects spatial memory loss in APPswe/PS1 Δ E9 mice (Hamilton et al., 2014). We now show that prolonged administration of CTEP not only rescues spatial memory deficits but also corrects deficits in episodic and recognition memory that are manifested earlier in APPswe/PS1 Δ E9 mouse disease progression. The observation that genetic deletion of mGluR5 corrects spatial memory impairment and reduces A β oligomer levels potentially could be dismissed as simply being the consequence of adaptations that may occur in the absence of mGluR5 during brain development. However, our findings that pharmacological intervention with CTEP in fully developed mice at 9 months prevents the progression and/or reverses memory impairment in APPswe/PS1 Δ E9 mice suggests that this outcome is not the consequence of a developmental

adaptation process. Rather, the pharmacological data indicate that mGluR5 is a centrally important contributor to AD-like disease cognitive impairment and pathology and that blockade of mGluR5 signaling with a negative allosteric modulator is neuroprotective. This conclusion is further substantiated by the observation that MTEP treatment of APP^{swe}/PS1 Δ E9 mice also improves performance in the MWM and can result in improved long-term potentiation (LTP) and reduced long-term synaptic depression (LTD) (Shankar et al., 2008; Li et al., 2009; Rammes et al., 2011; Um et al., 2013). Moreover, antagonists of mGluR5 prevent the block of LTP mediated by A β oligomers (Wang et al., 2004). Thus, it is possible that blockade of mGluR5 with CTEP improves cognition by reducing mGluR5-mediated LTD induced by A β oligomers, and the reduction of AD pathology may occur as a consequence of reduced mGluR5-dependent APP translation.

Of particular mechanistic interest to AD disease progression is the finding that both genetic deletion and chronic pharmacological blockade reduce both A β oligomer levels and the formation of A β plaques (Hamilton et al., 2014). This surprising and unexpected result indicates that mGluR5 signaling contributes directly to the establishment of AD-like disease pathology in APP^{swe}/PS1 Δ E9 mice. It is reported that A β oligomers promote mGluR5 clustering at synapses, resulting in increased activation of Ca²⁺ signaling, and A β oligomer-activated mGluR5 signaling may be associated with accentuated LTD (Renner et al., 2010; Hu et al., 2014). Thus, it is likely that CTEP not only functions to block glutamate-mediated activation of mGluR5 but also pathological A β oligomer-mediated mGluR5 signaling contributes directly to AD-like pathology linked to impaired cognitive behavior, as we observe an amelioration of AD pathology in both APP^{swe}/PS1 Δ E9 and 3xTg-AD mice. This suggests that the ongoing activation of mGluR5 by A β 42 oligomers may result in a growing pathological insult that can be prevented by long-term mGluR5 antagonism. Thus, although under normal physiological conditions mGluR5 blockade is not desirable, under pathophysiological conditions where mGluR5 is engaged by a non-physiological ligand (A β oligomers), mGluR5 antagonism results in a beneficial physiological effect by reversing an undesired pathophysiological signal.

Surprisingly, either mGluR5 antagonism or knockout corrects the cognitive impairment normally observed in APP^{swe}/PS1 Δ E9 mice, despite previous observations that mGluR5 contributes to processes associated with memory and learning (Lu et al., 1997; Hamilton et al., 2014; Hu et al., 2014). However, consistent with the fact that mGluR5 is important for memory and learning, we find that CTEP treatment does cause a discernible impairment in spatial memory tasks in the MWM in wild-type mice. Thus, under normal physiological conditions, it is likely that mGluR5 blockade is not desirable. But, under pathophysiological conditions where mGluR5 is engaged by a non-physiological ligand, such as A β oligomers, antagonism of the receptor likely results in a beneficial physiological effect that antagonizes the outcome of a pathophysiological signal.

In conclusion, CTEP is currently in pre-clinical development for the treatment of major depression disorder and fragile X syndrome, and it has been utilized successfully to treat the symptoms of preclinical mouse models of major depression dis-

order and fragile X syndrome (Michalon et al., 2012; Lindemann et al., 2015). We have now provided both genetic and pharmacological evidence that mGluR5 is an ideally suited target to ameliorate the clinical progression of AD. Thus, based on the desirable bioavailability and selectivity of CTEP, and that its analogue Basmiglurant is in phase II clinical trials, we suggest mGluR5 NAMs are ideal small molecule candidate drug for repurposing potential for clinical use in the treatment of AD.

EXPERIMENTAL PROCEDURES

Detailed experimental procedures are provided in the [Supplemental Experimental Procedures](#).

Animals

APP^{swe}/PS1 Δ E9 and 3xTg-AD mice were purchased from the Jackson Laboratory. All animal experiments were conducted in accordance with the University of Western Ontario and University of Ottawa animal care committees.

Novel Object Recognition

Time spent exploring each object was recorded. Mice were considered to be exploring an object if their snout was within 1 cm of the object. Then 3 hr after first exposure, one object was replaced with a novel object and mice were returned to the maze for 10 min. Data were interpreted using a recognition index, which was the percentage of time exploring the familiar object versus the novel object.

MWM and RMWM

Acquisition Phase, Days 1–4

Mice were trained over 4 consecutive days, four trials per day, with 15-min intervals between trials. Mice were randomly started from four equally spaced points around the pool, across each of the four daily trials. Animals were given 90 s to find the escape platform; if they failed to do so, they were guided to the platform where they remained for 30 s before being returned to their home cage. Swim speed and escape latency were recorded and statistical analysis was performed using GraphPad Prism.

Probe Trial, Day 5

The probe trial was performed to assess spatial memory by removing the platform and recording the time the mice spent in the target quadrant. RMWM was initiated 24 hr after the completion of MWM, and it was performed using the same paradigm as MWM, with 4 days acquisition and probe trial. In the RMWM task, the platform was moved to a new location. Analysis was performed as described for MWM.

A β Immunohistochemistry

Brains were coronally sectioned to include both the cortex and hippocampus, and analysis was performed on 40- μ m free-floating sections using a peroxidase-based immunostaining protocol. Sections were incubated in primary antibody for A β overnight at 4°C, washed, incubated in biotinylated antibody, and then incubated in an avidin biotin enzyme reagent. Sections were mounted on slides and visualized with a Zeiss LSM-510 META multiphoton laser-scanning microscope with a Zeiss 10 \times lens, using representative 900 \times 900- μ m areas of cortex and hippocampus.

Determination of A β Oligomer Concentration by Sandwich ELISA

In brief, brains were dissected into right and left hemispheres, with one hemisphere used to analyze oligomeric A β . Quantification of A β oligomers, in 12-month-old fresh mouse brains, was performed using a sandwich ELISA kit (KHB3491, Thermo Fisher Scientific) according to the manufacturer's instructions.

Statistical Analyses

Data in the recognition test were analyzed by 2 (strain) \times 2 (drug treatment), whereas the dependent outcomes for the MWM test were analyzed by a mixed-measures ANOVA.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2016.04.077>.

AUTHOR CONTRIBUTIONS

A.H. and S.S.G.F. designed the study and wrote the manuscript. S.S.G.F. supervised the study. A.H., M.V., C.V.T., R.J.M., and H.A. performed experiments and data analysis.

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