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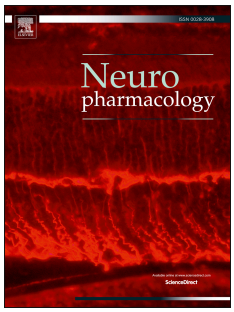

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Targeting glutamatergic and cellular prion protein mechanisms of amyloid ß-mediated persistent synaptic plasticity disruption: longitudinal studies

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Brief running title: Glutamate, Aß and LTP

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ABSTRACT

Alzheimer's disease amyloid-ß (Aß) oligomers are synaptotoxic, inappropriately increasing extracellular glutamate concentration and glutamate receptor activation to thereby rapidly disrupt synaptic plasticity. Thus, acutely promoting brain glutamate homeostasis with a blood-based scavenging system, glutamate-oxaloacetate transaminase (GOT), and blocking metabotropic glutamate 5 (mGlu5) receptor or its co-receptor cellular prion protein (PrP), prevent the acute inhibition of long-term potentiation (LTP) by exogenous Aß. Here, we evaluated the time course of the effects of such interventions in the persistent disruptive effects of Aß oligomers, either exogenously injected in wild type rats or endogenously generated in transgenic rats that model Alzheimer's disease amyloidosis. We report that repeated, but not acute, systemic administration of recombinant GOT type 1, with or without the glutamate co-substrate oxaloacetate, reversed the persistent deleterious effect of exogenous Aß on synaptic plasticity. Moreover, similar repetitive treatment reversibly abrogated the inhibition of LTP monitored longitudinally in freely behaving transgenic rats. Remarkably, brief repeated treatment with an mGlu5 receptor antagonist, basimglurant, or an antibody that prevents Aß oligomer binding to PrP, ICSM35, also had similar reversible ameliorative effects in the transgenic rat model. Overall, the present findings support the ongoing development of therapeutics for early Alzheimer's disease based on these complementary approaches.

Keywords: Alzheimer's disease; long-term potentiation; glutamate-oxalate transaminase; metabotropic glutamate receptor 5, *APP* transgenic rat.

1. Introduction

The, albeit relatively limited, clinical effectiveness of the NMDA receptor antagonist memantine in the treatment of Alzheimer's disease strongly supports the search for alternative glutamate-based therapeutic strategies (Deardorff and Grossberg, 2016). Consistent with a key role for glutamate-mediated synaptotoxic mechanisms, postmortem glutamatergic synaptic loss is a very strong morphological correlate of cognitive impairment in Alzheimer's disease (Davies et al., 1987; DeKosky and Scheff, 1990; Overk and Masliah, 2014; Terry et al., 1991). Indeed, there is now convincing evidence for a causal role for amyloid-ß (Aß) at the early stages of Alzheimer's disease and oligomeric Aß, which is a potent synaptotoxin, associates with disease (Bilousova et al., 2016; Cohen et al., 2015; Dohler et al., 2014; Lesne et al., 2013; Mc Donald et al., 2010).

In animal models of Alzheimer's disease amyloidosis there is growing evidence of functional glutamate overload and disruption of glutamate homeostasis (Rudy et al., 2015). The disturbance is likely caused by a combination of factors including excessive release of glutamate from glial cells and disinhibited neurons, impaired glutamate clearance by loss of functional glutamate transporters, and distorted glutamate receptor trafficking (Matos et al., 2008; Mucke and Selkoe, 2012; Renner et al., 2010; Talantova et al., 2013; Ulrich, 2015). Although blocking certain NMDA receptors has beneficial effects in many models, the deleterious and dose-limiting side effects of NMDA receptor antagonists are well documented (De Felice et al., 2007; Ronicke et al., 2011; Zadori et al., 2014). There is growing evidence of the potential superiority of targeting metabotropic glutamate rather than NMDA receptors, in particular antagonists selective for subtype 5 (mGlu5) (Haas et al., 2016; Hamilton et al., 2014; Hamilton et al., 2016; Lauren et al., 2009; Um et al., 2013; Viola and Klein, 2015). Importantly, some of these antagonists have been tested in humans,

displaying an apparently good safety profile (Berry-Kravis et al., 2016; Reilmann et al., 2015; Scharf et al., 2015; Tison et al., 2016).

Antagonists at the mGlu5 receptor not only block potential adverse effects of Aß-triggered excess glutamate but also prevent Aß oligomer-mediated aberrant mGlu5 receptor trafficking (Renner et al., 2010) and coupling to downstream effectors via binding to cellular prion protein (PrP) (Beraldo et al., 2016; Haas et al., 2016; Hamilton et al., 2015; Lauren et al., 2009; Um et al., 2013). Indeed, PrP is a receptor for certain synaptotoxic Aß oligomers and blocking the binding of Aß oligomers to PrP is also a potentially attractive option (Chung et al., 2010; Jarosz-Griffiths et al., 2016; Resenberger et al., 2011).

Alternative strategies being investigated to counter glutamate dyshomeostasis in Alzheimer's disease include agents that increase glutamate removal from the interstitial fluid in the brain directly by inducing glutamate transporters, or indirectly by increasing the metabolism of glutamate (Takahashi et al., 2015; Zhang et al., 2016).

Plasticity at glutamatergic synapses is extremely sensitive to disturbance by endogenously generated and exogenously applied synaptotoxic soluble Aß species (Cullen et al., 1997; Lambert et al., 1998; Randall et al., 2010; Shankar et al., 2008; Walsh et al., 2002), at least partly through mechanisms involving glutamate dyshomeostasis (Lei et al., 2015; Li et al., 2009). Previous studies of the acute effects of enzyme-mediated glutamate scavenger systems have shown potential beneficial effects against the acute plasticity disrupting effects of exogenously applied Aß both *in vitro* and *in vivo* (Li et al., 2009; Varga et al., 2015; Zhang et al., 2016). In the latter case a blood-based scavenger system consisting of glutamate oxaloacetate transaminase (GOT) with oxaloacetate (OXA) was found to transiently abrogate synthetic Aß and Alzheimer's disease brain Aß-mediated inhibition of long-term potentiation (LTP) in acutely anaesthetized rats. By increasing the conversion of glutamate in blood to α-

ketoglutarate, the efflux of excessive glutamate in brain is accelerated (Campos et al., 2011b; Castillo et al., 2016; Zlotnik et al., 2007).

Similar to agents that lower extracellular glutamate concentration in the brain, antagonists at glutamate receptors can prevent the acute disruption of synaptic plasticity by exogenously applied Aß (Hu et al., 2008; Li et al., 2009; Rammes et al., 2011; Wang et al., 2004). In particular, the mGlu5 receptor may be critical for both inhibition of LTP and facilitation of long-term depression (LTD) (Hu et al., 2014; Li et al., 2009; Ronicke et al., 2011; Wang et al., 2004). Furthermore, consistent with findings that cellular PrP acts as a co-receptor for mGlu5 receptors, blocking Aß binding to PrP prevents the acute disruption of synaptic plasticity by Aß (Barry et al., 2011; Freir et al., 2011; Hu et al., 2014; Klyubin et al., 2014a; Lauren et al., 2009; Nicoll et al., 2013)

Here we directly compare the time course and efficacy of blood based glutamate scavengers, mGlu5 receptor antagonists and an antibody preventing Aß binding to PrP, to ameliorate Aß-mediated persistent disruption of synaptic plasticity *in vivo.* We examined the persistent inhibition of LTP caused by either exogenous i.c.v. application of a synthetic Aß1-42 oligomer-enriched preparation or endogenous Aß in a very complete APP-based transgenic (TG) rat model of amyloidosis (Leon et al., 2010). In longitudinal studies in freely behaving TG rats the reversal of the synaptic plasticity deficits caused by repeated treatment with these agents was monitored in individual animals.

2. Material and Methods

2.1 Animals and surgery

Rats were housed under a 12 h light-dark cycle at room temperature (19-22 °C). The animal care and experimental protocol were approved by the Department of Health, Republic of Ireland and the Irish Health Products Regulatory Authority in accordance with EU law.

The effects of Aß were studied in adult (250-350 g, 8-13 weeks old) male Wistar and Lister Hooded rats. In order to record synaptic transmission the surgical and electrophysiological methods were similar to those used previously (Hu et al., 2014). For electrophysiolgical recording anaesthesia was induced and maintained with urethane (1.5-1.6 g/kg, i.p.). Monopolar recording electrodes (75 μm inner core diameter, 112 μm external diameter) (3.4 mm posterior to bregma and 2.5 mm lateral to midline) and twisted bipolar stimulating electrodes (50 μm inner core diameter, 75 μm external diameter) (4.2 mm posterior to bregma and 3.8 mm lateral to midline) were constructed from Teflon coated tungsten wires. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum in the CA1 area of the dorsal hippocampus in response to stimulation of the ipsilateral Schaffer collateral-commissural pathway. The final placement of electrodes was optimized by using electrophysiological criteria and confirmed via post-mortem analysis.

In order to inject Aß or antibody into the brain, a stainless-steel cannula (22 gauge, 0.7 mm outer diameter) was implanted ipsilaterally above the right lateral ventricle (1 mm lateral to the midline and 4 mm below the surface of the dura). The solution was injected intracerebroventricularly (i.c.v.) via an internal cannula (28 gauge, 0.36 mm outer diameter) at a rate of 1-2 μl per min with a Hamilton syringe (Reno, Nevada, USA). For Aß injection the cannula was temporarily implanted under recovery anaesthesia using a mixture of ketamine and xylazine (80 and 8 mg/kg, respectively, i.p.). Following the i.c.v. injection of Aß or vehicle the guide cannula was removed. The day of i.c.v. injection was termed day 0, following which, these animals were housed individually in their home cages. Seven to

fourteen days later, the animals were implanted and recorded under non-recovery anesthesia, as described above.

In the studies of synaptic plasticity disruptive effects of endogenous Aß adult male TG rats expressing human *APP751* with Swedish and Indiana mutations under the control of the murine *Thy1.2* promoter (McGill-R-Thy1-APP) (Iulita et al., 2014; Leon et al., 2010) and their age-matched wild type (WT) littermates were studied. The TG animals were genotyped as outlined in (Qi et al., 2014) and ages varied from 3 to 13 months old.

For the chronic recording the implantation procedure was carried out under anaesthesia using a mixture of ketamine (80 mg/kg) and xylazine (8 mg/kg) (both i.p.) according to methods similar to those described previously and is comparable to that described above (Li et al., 2003). The recording site was located 3.8 mm posterior to bregma and 2.5 mm lateral to midline, and the stimulating site was located 4.6 mm posterior to bregma and 3.8 mm lateral to midline. All chronically implanted animals also had a cannula inserted over the lateral ventricle, as described above. These rats were allowed at least 14 days after surgery before recordings began and were housed individually in their home cages between recording sessions.

2.2 Stimulation and recording

Test EPSPs were evoked by square wave pulses (0.2 ms duration) at a frequency of 0.033 Hz and an intensity that triggered a 50% maximum response as determined after constructing an input/output curve. Baseline synaptic transmission was recorded for at least 30 min. In some experiments paired-pulse stimulation was applied using a 40 ms inter-pulse interval.

Awake recordings in the recovery animals were carried out in a well-lit room. The recording compartment consisted of the base of the home cage, including normal bedding and

food/water, but the sides were replaced with a translucent Perspex plastic box (27× 22× 30 cm) with an open roof. The rats had access to food and water throughout the whole recording session from the same position as in the home cage. All animals were first habituated to the recording procedure over the post-surgery recovery period.

LTP was induced using 200 Hz high frequency stimulation (HFS) consisting of 10 trains of 20 stimuli with an inter-train interval of 2 s. A single series was used in the awake rat recordings whereas, apart from in Fig. 1, three sets (inter-set interval 5 min) were applied in the anaesthetized rats. The stimulation intensity was not changed during HFS.

2.3 Preparation of synthetic Aß-derived diffusible ligands (ADDLs)

An Aß solution enriched with oligomers including protofibrils (ADDLs) was prepared using synthetic Aß1-42. Aβ1–42 was synthesized and purified by the ERI Amyloid laboratory Oxford, CT, USA. Two slightly different methods were used. For the electrophysiology experiments, as described previously (Hu et al., 2014), a 1 mM solution of Aß1–42 was prepared in hexafluoroisopropanol and incubated at 37 °C for 1 h and briefly vortexed every 10 min. The hexafluoroisopropanol was evaporated using a speedvac and the dried peptide film stored overnight over desiccant at −20 °C. A peptide solution of 22.5 mg/ml was then prepared in anhydrous dimethylsulfoxide (DMSO) and subsequently diluted 1:50 with Hams F-12 media. After incubation at 4 °C for ~14 h the solution was centrifuged at 16,000×g for 10 min. The supernatant was recovered and absorbance at 275 nm recorded. The concentration of oligomeric Aß was determined using size exclusion chromatography. Aliquots of ADDLs were frozen on dry ice and stored at −80 °C until needed. For standardizing the DELFIA (dissociation-enhanced lanthanide fluorescence immunoassay), ~25 mg peptide was dissolved in 2% w/v anhydrous DMSO and gently rocked for ~ 5 minutes, then diluted to 0.5 mg/ml in phenol red-free Ham's F12 medium without L-

glutamine (Caisson Labs) and incubated quiescently at room temperature. Aggregation was monitored using asymmetric field flow fractionation. Aβ oligomer formation was judged to be complete when <20% of the injected mass eluted as monomer, typically after 24 - 36 h incubation. The ADDLs were further assessed by negative-stain electron microscopy. Depending on the batch of ADDLs, the amount injected was either 480 or 585 pmol (in 5-12 µl).

2.4 Drugs and chemicals

Oxaloacetate was purchased from Sigma-Aldrich, Wicklow, Ireland. The oxaloacetate dose chosen (35 mg/kg in 1 ml, i.v.) was based on previous research (Perez-Mato et al., 2014). The dose of recombinant glutamate-oxaloacetate transaminase type 1 (rGOT) (Megazyme, Bray, Ireland) given (0.39 mg/rat i.v.) was previously reported to significantly increase GOT serum concentration for over 20 h (Ruban et al., 2015). Treatment (s.c.) with rGOT started immediately after i.c.v. Aß using a dose of 0.39 mg/rat, followed by a maintenance dose of 0.13 mg/rat/day to day 6 at 24 h intervals.

In the awake animal recordings, the co-administration of OXA (35mg/kg) and rGOT (0.39 mg/rat) or vehicle (saline, 1 ml) was given i.v. via tail vein to TG rats and WT littermates under transient recovery anaethesia (2% isoflurane). After full recovery, the rats were transported to the recording cage and then recording was initiated. **How was rGOT given?**

Basimglurant and 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-imidazol-4-yl)ethynyl)pyridine (CTEP) (both DC chemicals, Shanghai, China), were initially dissolved in 10% DMSO (Sigma, Dorset, UK), and administered as a suspension in corn oil (volume 0.3-0.4 ml) vehicle. A dose of 2 or 3 mg/kg i.p. was chosen to obtain high mGlu5 receptor occupation (Jaeschke et al., 2015; Lindemann et al., 2011; Lindemann et al., 2015).

For repeated dosing we chose a 3 day schedule, which had proven effective in preventing LTP deficits in the APP transgenic rats using different anti-Aß interventions (Qi et al., 2014). In preliminary studies a lower dose of 0.4 mg/kg (twice over 3 days, i.p.) failed to significantly reverse the LTP deficit (n=3; data not shown). Because of their different half-lives basimglurant was injected once daily whereas CTEP was administered twice, on day 1 and 3. The last injection was 2 h before application of HFS.

The anti-PrP antibody ICSM35 and isotope control antibody BRIC126 were stored as stock solutions of 6mg/ml in phosphate-buffered saline (PBS). Injections (10 µl over 3-5 min) were made via a Hamilton syringe connected to the internal cannula. Based on pilot studies and our previous studies (Qi et al., 2014) of the effect of the anti-Aß monoclonal antibody McSA1, we chose a protocol of 5 injections of 60 µg, over 3 days, the 5^{th} injection being carried out at 2 h before application of HFS.

2.5 Homogenisation of rat brain tissue for Aß oligomer DELFIA

Hemi-brains from TG and WT rats varying in age from 3-13 months were frozen at -80 ^{o}C until needed for Aß oligomer ELISA. All of the genotypes of the rat brain hemispheres were anonymized, and then homogenized using a Precellys-24 Ribolyser in Dulbecco's PBS containing Roche Complete protease inhibitor to a final concentration of 10% (w/v). The homogenates were assayed for total protein concentration using the Bradford assay by two different users until a percentage coefficient of variation less than 10% per sample between users was obtained. The homogenates were aliquoted into single thaw volumes in low binding eppendorfs (Fisher Scientific).

2.6 Detection of Aβ oligomers using the anti-Aβ oligomer antibody 1C22

The anti-Aβ oligomer antibody 1C22 is a monoclonal murine antibody that binds strongly to Aβ oligomers but only weakly to Aβ monomers (Yang et al., 2015). 1C22 (30 µl at 23 µg/ml in 10 mM sodium carbonate, pH 9.6) was immobilised on a Lumitrac high binding 384 well plate (Greiner Bio-one #781074). The plate was incubated at 400 rpm for 1 h at 37 °C then washed. All washes were performed with three 100 µl per well washes of PBS (0.05% Tween-20). The plate was blocked with 100 µl per well Superblock (Thermo Scientific) with shaking at 400 rpm at 37 °C for 2 h and washed. The rat brain homogenates were defrosted, benzonase treated, and diluted in Dulbecco's PBS to give equivalent total protein concentrations (as assessed by the Bradford assay). Additionally, a serial dilution of synthetic ADDLs was prepared in age-matched wild-type rat brain homogenate to produce the standard curve for each experiment. Aliquots of 30 µl of brain homogenates and standards were incubated in the plate for 1 h at 25 °C with shaking at 400 rpm followed by a plate wash. Aβ was detected by 30 µl of 0.2 µg/ml biotinylated 82E1 in DELFIA assay buffer (PerkinElmer) for 1 h at 25 °C with shaking at 400 rpm. The plate was washed then incubated for 1 h at 25 °C with shaking at 400 rpm with 333 ng/ml of DELFIA Eu-N1 streptavidin antibody in DELFIA assay buffer (PerkinElmer), washed before enhancing with 80 µl of DELFIA Enhancement Solution (PerkinElmer) for 10 min. Plates were scanned for time-resolved fluorescence intensity of the europium probe (λ_{ex} 320 nm, λ_{em} 615 nm) using a PerkinElmer EnVision plate reader. All samples were run in quadruplicate.

The standard curve obtained from the serial dilution of ADDLs was used to convert the time resolved fluorescence intensity of each of the brain homogenates into the concentration of Aβ oligomers. The standard curve was also used to determine the lower limit of quantification (LLOQ) defined as the lowest standard with a percentage backfit of 100±20% and a percentage coefficient of variation of <20% and over 9 times the standard deviations of negative blank signal.

2.7 Serum glutamate assay

Blood samples were collected in test tubes, centrifuged at 600×g for 10 min, serum was removed and immediately frozen and stored at –80 °C. Serum glutamate concentration was determined by means of a glutamate assay kit (Cat. No. MAK004, Sigma-Aldrich, Wicklow, Ireland) following the manufacturer's technical instructions.

2.8 Statistical analysis

The strength of synaptic transmission is expressed as a percentage of the baseline fEPSP amplitude recorded over at least a 30 min period. The magnitude of LTP was measured at 3 h post-HFS and expressed as the mean ± SEM % baseline. No data were excluded. Control experiments were interleaved randomly throughout experimental sets. Sample sizes including animal numbers were chosen to ensure adequate statistical power comparable to previously published papers. Data distribution of experiments was assumed to be normal. For statistical analysis and graphical display EPSP amplitude was grouped into 10 min epochs. One-way ANOVA followed by Bonferroni's multiple comparison test was used to compare the magnitude of LTP between multiple groups; paired and unpaired Student's *t*-tests were used to compare within and between groups, respectively. Serum glutamate concentration is expressed as the mean ± SEM and analysed statistically in a similar manner. The levels of Aß oligomers in TG rats were assessed by Fisher's exact test and Pearson's r. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Acute treatment with blood-based glutamate scavengers fails to reverse synthetic Aß-mediated persistent disruption of synaptic plasticity recorded in anaesthetized rats

The ability of the peripherally acting glutamate scavenger oxaloacetate to reverse the synaptic plasticity disrupting action of Aß was first tested in a delayed model. Previously, soluble extract of Alzheimer's disease brain that contains Aß was reported to persistently inhibit LTP, without significantly affecting baseline synaptic transmission, after a single i.c.v. injection (Klyubin et al., 2014b). Here, an ADDL preparation of synthetic Aß1-42 (i.c.v.) was injected under recovery anaesthesia and the ability to induce LTP was determined 7-9 or 14 d later under non-recovery anaesthesia. Whereas the application of HFS failed to induce LTP in the animals tested on d 7-9 (Aß7, 107.4±5.4%, mean ± SEM% pre-injection baseline EPSP amplitude at 3 h post-HFS, n=8; $p<0.05$ compared with 132.6±4.8% in controls, n=8, ANOVA followed by Bonferroni test), in animals injected i.c.v. with Aß 14 d previously the magnitude of LTP was comparable to control levels (Aß14, 131.6±3.5; $p>0.05$) (Fig. 1A, B). The inhibition of LTP appeared to be relatively selective since there was no significant effect of Aß injection on baseline excitatory transmission as measured by the amplitude of the fEPSP (maximum fEPSP amplitude: vehicle group, 4.7±0.5 mV; Aß7 group, 4.4±0.5 mV; Aß14 group, 5.3±0.5 mV; $p>0.05$), and short-term plasticity as measured by the level of paired-pulse facilitation at a 40 ms inter-pulse interval (paired-pulse ratio: vehicle group, 1.49±0.06; Aß7 group, 1.64±0.18; Aß14 group, 1.49±0.07; $p>0.05$) (Fig. 1C, D).

Initially we studied the effect of oxaloacetate using a single bolus injection of a dose (35 mg/kg, i.v.) that previously has been reported to reduce free blood and brain glutamate concentration (Campos et al., 2011b; Perez-Mato et al., 2014). We previously reported that this intervention fully prevented the acute disruption of hippocampal synaptic plasticity caused by Aß and reduced blood glutamate concentration by ~30-50% when measured 30 min and 1 h after injection (Zhang et al., 2016). In the present studies, however, the delayed LTP inhibition by Aß injection 7 d previously was not significantly affected by a single i.v. injection of oxaloacetate under non-recovery anaesthesia. Similar to above, in the Aß group

that received an i.v. injection of saline vehicle, the application of HFS failed to induce LTP (106.1±6.7%, n=6; $p<0.05$ compared with 137.6±2.1% in controls that received i.c.v. and i.v. vehicle injections, n=5, ANOVA followed by Bonferroni test) (Fig. 2A, D). Moreover Aß also blocked LTP in animals that received an i.v. injection of oxaloacetate either 20 min or 1 h before the application of HFS (20 min pre-injection: 110.6±4.0%, n=5 data not shown; $p>0.05$ compared with 106.1±6.7%, n=6 in Aß+vehicle,; 1 h pre-injection: 116.8±4.6%, n=5; $p>0.05$ compared with 109.7±5.5, n=5, in animals that received Aß followed by vehicle) (Fig. 2B,E).

Next we attempted to enhance the blood-scavenging effect of oxaloacetate by increasing the blood concentration of GOT using recombinant protein type 1 (rGOT) (Perez-Mato et al., 2014; Ruban et al., 2015; Zlotnik et al., 2007). We previously reported that co-treatment with rGOT (0.39 mg/rat) prolonged the oxaloacetate-mediated reduction in serum glutamate clearance and the abrogation of the acute deleterious effect of Aß on LTP (Zhang et al., 2016). However, in animals that received Aß (i.c.v.) 7 d previously, neither rGOT alone nor the co-administration of rGOT and oxaloacetate reversed the delayed disruption of LTP when applied 1 h prior to the HFS (117.0±1.4, n=5 and 108.0±6.0%, n=5 respectively; $p>0.05$ compared with pre-HFS baseline or compared with Aß+vehicle) (Fig. 2B,C,E).

3.2. Repeated systemic treatment with blood-based glutamate scavengers reverse synthetic Aß-mediated persistent disruption of synaptic plasticity

We hypothesized that the apparent lack of efficacy of acute treatment with the blood glutamate scavenging interventions against the delayed inhibition of LTP by Aß given 7 d before may be due to a gradual build up of excess glutamate during the post-Aß injection. Previously, repeated subcutaneous (s.c.) injection of rGOT was reported to persistently increase the blood levels of GOT and to exert a neuroprotective effect against glutamate

excitotoxicity in an amyotrophic lateral sclerosis model (Ruban et al., 2015). Therefore in addition to acutely injecting blood-based glutamate scavengers i.v. on day 7, we repeatedly applied rGOT for 7 d (0.39 mg/rat, s.c on the day of Aß administration, day 0, followed by a daily maintenance dose of 0.13 mg/rat on days 1-6). In animals pre-treated with i.c.v., Aß followed by control vehicle systemic injections, LTP was strongly inhibited (107.1±3.7%, n=6; p<0.05 compared with 128.5±1.6%, n=6, in the control group, which received vehicle for the i.c.v., s.c. and i.v injections) (Fig. 3A, C). Importantly, repeated daily s.c. injection of rGOT followed on day 7 with an acute i.v. injection of rGOT (0.39 mg/rat), either with or without oxaloacetate (35 mg/kg), abrogated the delayed synaptic disruption by Aß given 7 d previously (121.5±1.6% and 129.2±3.6%, respectively, n=6 per group; p<0.05 compared with the Aß+vehicle group; p>0.05 compared with vehicle control group) (Fig. 3B, C). Although the addition of oxaloacetate on day 7 appeared to enhance the beneficial effect of GOT treatment, there was no significant difference between the Aß+rGOT+veh/rGOT and Aß+rGOT+OXA/rGOT groups (p>0.05). Moreover, the combination treatment schedule did not significantly affect the magnitude of control LTP (133.3±2.6%, n=5; p>0.05 compared with vehicle control group).

Consistent with the electrophysiological findings, both of the effective treatment protocols significantly decreased serum glutamate concentration to similar extents (65.4±5.9 and 64.1±9.7 μM, for rGOT s.c. followed by an i.v. injection of either rGOT alone or in combination with oxaloacetate, respectively, n=5 per group; p<0.05 compared with 137.7±21.1 μM in samples taken from a vehicle control group, n=5) at 1 h post-i.v. injection, the time point when HFS was applied (Fig. 3D).

3.3 Both acute and repeated metabotropic glutamate 5 receptor antagonist treatment reverses synthetic Aß-mediated persistent disruption of synaptic plasticity

The efficacy of repeated treatment with blood-based glutamate scavengers to abrogate the persistent inhibition of LTP by Aß is likely due to indirectly reducing excessive brain levels of glutamate. Therefore we hypothesized that centrally acting antagonism of mGlu5 receptors, one of the main mediators of acute synaptic plasticity disruption by Aß, would also be effective in reversing the persistent inhibition of LTP. We gave the selective mGlu5 receptor negative allosteric modulator basimglurant, which acts as a non-competitive antagonist of the receptor i.p. (2 mg/kg, either acutely or 3 times at 24 h interval with the last injection given 2 h prior to HFS). Either the acute or repeated treatment with basimglurant reversed the inhibition of LTP. Thus application of HFS 2 h after acute treatment triggered robust LTP (145.1±8.1% at 3 h post-HFS, $p>0.05$ compared with 89.5±18.5% in Aß pre-injected rats, n=4 per group) (Fig. 4A,D). Similarly, repeated injection on days 5-7 of basimglurant abrogated the persistent inhibition of LTP caused by i.c.v. injection of Aß 7 days previously (120.5±4.8% n=5; $p<0.05$ compared with 96.7±5.0% in the Aß+vehicle group, n=6) (Fig. 4B,E). This repeated basimglurant treatment regime had no significant effect on control LTP (128.9±4.0% n=5, $p>0.05$ compared with 131.4±3.7% n=5 in vehicle control group) (Fig. 4C,F).

3.4 Acute treatment with blood-based glutamate scavengers fail to reverse the disruption of synaptic plasticity in freely behaving transgenic rats modeling Alzheimer's disease amyloidosis

In order to determine if agents that lower blood glutamate levels can reverse synaptic plasticity deficits caused by endogenously produced human Aß, we tested their effectiveness in preventing LTP impairment in freely behaving rats overexpressing APP. Rats expressing human *APP751* with Swedish and Indiana mutations (McGill-R-Thy1-APP, TG) have an age-dependent, Aß-mediated, impairment in LTP induction compared with age-matched WT littermates (Qi et al, 2014).

To establish if and when TG rats accumulate pathologically relevant Aβ aggregates we employed a variant of the highly sensitive Aβ oligomer-specific immunoassay developed to quantify soluble Aβ aggregates in AD brain (Yang et al., 2015). Aβ oligomers were not detected in any WT animals tested (n=10, age range 3 – 13 months). In contrast, at 3-13 months of age there were significantly more TG animals with Aβ oligomer level above the lower limit of quantification (LLOQ, 80 pM) (11 of 28 TG rats, $p<0.05$ compared with WT rats, Fisher's exact test). Indeed Aβ oligomers were detected in TG rats as young as 4 months (Fig. 5A). Whereas at 3 - 7 months of age oligomers were detectable in 7 out of 23 TG rats, at 12 – 13 months of age 4 out of 5 TG animals had detectable amounts of Aβ oligomers. Thus there was a highly significant correlation between age and Aβ oligomer level of TG rats with readings above the LLOQ (n=17, Pearson r=0.89, $p<0.05$, R squared=0.79).

By studying chronically implanted animals it was possible to carry out both cross-sectional and longitudinal analyses of the treatments. We studied a range of ages just before or at the start of plaque deposition (6-10 month old).

First we confirmed that this group of TG rats had a deficit in LTP (103.0±4.6% n=6, $p<0.05$ compared with 142.5±7.0% n=6 in WT litter mate group) (Fig. 5B, D). Next we examined the effect of acute treatment with the blood-based glutamate scavengers. Co-administration (i.v.) of oxaloacetate and rGOT as a single bolus injection 1 h prior to HFS did not appear to affect control LTP in WT littermates (134.0±4.7% n=6, $p<0.05$ compared with pre-HFS baseline) or the LTP deficit in TG rats (112.3±6.9% n=6, $p>0.05$ compared with pre-HFS baseline, $p<0.05$ compared with WT) (Fig. 5 C, E).

3.5 Repeated systemic treatment with blood-based glutamate scavengers reverse the disruption of synaptic plasticity in freely behaving transgenic rats modeling Alzheimer's disease amyloidosis

In view of the ability of repeated administration of blood-based glutamate scavengers to abrogate the persistent inhibition of LTP by exogenous Aß, next we studied the effect of the same rGOT and oxaloacetate treatment schedule on the LTP deficit in amyloid precursor protein (APP) TG rats. Hence, repeated subcutaneous (s.c.) injection of rGOT (0.39 mg/rat initially, and a daily maintenance dose of 0.13 mg/rat for 7 d), was followed by an acute (i.v.) co-injection rGOT and oxaloacetate at 1 h prior to HFS on day 7. The ability to induce LTP was tracked longitudinally before, during and after treatment.

Consistent with our hypothesis, in drug treated TG rats HFS induced robust LTP (130.5±2.3%, n=5, $p<0.05$ compared with 107.4±2.8%, n=5 in the TG rats that received vehicle treatment) (Fig. 6A, B). We also carried out a longitudinal analysis. As can be seen in Fig. 5C, D, HFS failed to induce persistent LTP when applied either just before initiating drug treatment or 7 days after treatment (99.2±3.1% and 109.7±7.8%, respectively, n=5; $p>0.05$). In the WT littermates, the repeated rGOT s.c. injection combined with acute OXA/rGOT i.v. injection did not significantly affect LTP (134.2±3.7% n=6 $p>0.05$ compared with 135.6±1.0% n=6 in the vehicle treated control group) (Fig. 6E, F). Moreover, in the longitudinal analysis it can be seen that the magnitude of LTP did not significantly change between pre-, during and post-treatment recording sessions (131.8±2.5% at 1 week post-treatment, $p>0.05$ compared with 142.5±7.0% n=6 in the same animals prior to treatment, n=6) (Fig. 6G, H).

3.6 Repeated systemic treatment with metabotropic glutamate 5 receptor antagonists rapidly reverses the disruption of synaptic plasticity in freely behaving transgenic rats modeling Alzheimer's disease amyloidosis

For comparison with the study that examined the effect of acute treatment with OXA/rGOT in McGill-R-Thy1-APP TG rats we tested the effect of a single injection of the mGlu5

receptor antagonist basimglurant on the ability to induce LTP in freely behaving animals. Similar to acute OXA/rGOT treatment, acute treatment with basimglurant failed to significantly reverse the LTP deficit in 5 month old TG rats. Thus, application of HFS 2 h after i.p. injection of a dose of 2 mg/kg did not induce significant LTP (99.3±2.8%, n=5, $p>0.05$ compared with 100.3±4.6% in the same animals one week prior to drug testing) (Fig. 6A-C). Somewhat similarly, in 5 month old TG animals that were tested 16 h after a dose of 3 mg/kg the magnitude of LTP was not significant at 3 h (102.7±3.5%, n=5, $p>0.05$ compared with 108.6±5.4% in the same animals one week prior to drug testing), although it appeared to decay more slowly in these animals (Fig. 6D-F).

As explained in the Methods section, because we had previously reported that 3 day treatment with anti-Aß interventions reversed the LTP deficit in the transgenic rat model (Qi et al., 2014) we also examined the effects of similar treatment with mGlu5 antagonists. Three-day treatment with basimglurant (2 mg/kg/day, i.p.) caused a strong but transient reversal of the LTP deficit in 5 month old TG rats. Thus, in the week prior to initiating drug treatment, application of HFS only induced a decremental potentiation (101.6±3.3% n=6 at 3 h post-HFS, $p>0.05$ compared with pre-HFS baseline, paired *t*-test). In contrast, 2 h after the third daily injection of basimglurant, the same HFS protocol now triggered robust and stable LTP in the same animals (120.8±3.3%, n=6, $p<0.05$ compared with pre-HFS baseline, paired *t*-test). However, seven days after the last injection of basimglurant, the LTP deficit had returned since HFS now only induced a decremental potentiation (102.0±1.9%, n=6, $p>0.05$ compared with pre-HFS baseline, paired *t*-test) (Fig 6).

In order to determine if the rapidly reversible beneficial effects of basimglurant were a drug class effect not caused by the vehicle treatment we decided to compare the effects of corn oil with another mGlu5 receptor antagonist, CTEP (Hamilton et al., 2016; Jaeschke et al., 2015; Lindemann et al., 2011). Treatment of 5 month old TG rats with CTEP (2 mg/kg

over 3 days, i.p.) caused a rapid and transient reversal of the LTP deficit (Fig. 7). Thus, in the week prior to initiating drug treatment, application of HFS only induced a decremental potentiation (98.4±1.6% n=5 at 3 h post-HFS, $p>0.05$ compared with pre-HFS baseline, paired *t*-test). In contrast, 2 h after the second injection of CTEP, the same HFS protocol now triggered robust and stable LTP in the same animals (125.5±2.4% n=5, $p<0.05$ compared with 100.4±2.0% n=5 in corn oil vehicle control). Moreover, seven days after the last injection of CTEP, the LTP deficit had returned since HFS now only induced a decremental potentiation (100.8±1.5%, n=5, $p>0.05$ compared with pre-HFS baseline, paired *t*-test).

3.7 An antibody that prevents Aß oligomer binding to cellular prion protein restores LTP in freely behaving transgenic rats modeling Alzheimer's disease amyloidosis

Previously, we and others reported that the acute inhibition of LTP by synthetic Aβ aggregates is prevented by acute pretreatment with antibodies against PrP that block, but not by those that do not affect, high affinity Aβ oligomer binding (Barry et al., 2011; Freir et al., 2011; Hu et al., 2014; Klyubin et al., 2014a; Lauren et al., 2009; Nicoll et al., 2013). Here we employed the monoclonal antibody ICSM35 which is directed against the Aβ oligomer-binding PrP 95-110 region (Freir et al., 2011; Khalili-Shirazi et al., 2007). In a blind study (Fig. 8), we found that repeated i.c.v. injections of ICSM35 (5x60 μg over 3 days), reversed the LTP deficit in 4.5-5 month-old freely behaving TG animals (131.0±5.6%, n=5, $p<0.05$, compared with 104.4±7.2%, in TG animals of similar age treated with an isotype control antibody, Bric126, n=6). Notably, full reversal of the effect of ICSM35 had not occurred by 2 weeks after ceasing treatment. Thus HFS applied 2 weeks after injections of ICSM35 (post) still induced LTP in 3 of the 5 rats (Fig. 8).

4. Discussion

Repeated systemic treatment with agents that reduce either excess glutamate, activation of mGlu5 receptors or binding to cellular PrP were effective in reversing persistent synaptic plasticity disruption caused by Aß oligomers. Administration of the blood-based glutamate scavenger rGOT alone or in combination with oxaloacetate lowered blood glutamate concentration and overcame the inhibition of LTP both by exogenously applied synthetic Aß1-42 aggregates in WT rats or endogenously generated Aß in pre-plaque transgenic rats. Moreover, brief repeated treatment with the mGlu5 receptor antagonists basimglurant and CTEP, or the PrP antibody ICSM35, exerted similar beneficial effects. Longitudinal monitoring of individual freely behaving transgenic rats revealed that the enhancement of LTP caused by these interventions is also relatively rapidly reversible. These data strongly indicate that the persistent impairment of LTP by either exogenous or endogenous Aß oligomers is mediated by excessively increased brain glutamate, inappropriate mGlu5 receptor activation and Aß oligomer binding to cellular PrP. In addition, the findings are potentially relevant to the development of clinical interventions designed to reverse synaptic deficits in patients with early Alzheimer's disease.

Blood-based glutamate scavengers offer a novel means of promoting brain glutamate homeostasis. The prediction, based on our previous studies on the abrogation of the acute inhibition of LTP by soluble synthetic or Alzheimer's disease brain Aß (Zhang et al., 2016), that acute treatment with the blood-based scavengers would ameliorate the persistent disruption of LTP by Aß was not supported. However, supplementation of the acute injections with repeated systemic treatment of rGOT was very effective in reversing the persistent inhibition of LTP caused by either exogenous or endogenous Aß exposure. Physiological homeostasis of glutamate concentration in the body is promoted by the blood resident enzyme GOT, which converts glutamate to α-ketoglutarate in the presence of oxaloacetate (Campos et al., 2011b; Castillo et al., 2016; Zlotnik et al., 2007). In the present

experiments this system was supplemented by systemic injection of both oxaloacetate and rGOT, neither of which cross the blood brain barrier to any significant extent (Boyko et al., 2012; Hassel et al., 2002). Blood-based glutamate scavengers preferentially promote excess glutamate clearance from the brain by increasing the concentration gradient between the blood and luminal side of endothelial cells in cerebral blood vessels, which in turn promotes removal of any excess glutamate in the brain's interstitial fluid (Castillo et al., 2016; Cohen-Kashi-Malina et al., 2012; Gottlieb et al., 2003; Ruban et al., 2015). The effects of oxaloacetate and rGOT on brain glutamate concentration should be selective for excessive levels, with minimal effects on physiological levels. This is because under physiological conditions glutamate is cleared primarily by neuronal and glial transporters and therefore would not reach sufficient levels to activate transporters on the abluminal side of the endothelial cells (Castillo et al., 2016; Gottlieb et al., 2003; Zlotnik et al., 2007). In the present studies it is unclear whether or not the success of supplementation with daily s.c. rGOT treatments was principally due to a boosting of the brain glutamate lowering effect of the acute i.v. injections or also because prolonged blood-based glutamate scavenging by rGOT is necessary to prevent persistent downstream deleterious effects of elevated brain glutamate concentration that mediate Aß-induced inhibition of LTP.

The ability of repeated systemic treatment with an mGlu5 receptor antagonist to mimic the ameliorative effect of the blood-based glutamate scavengers in the two chronic Aß models lends support to the critical role of inappropriate glutamate receptor activation in mediating the persistent inhibition of LTP by exogenous and endogenous Aß. These data extend previous studies on the efficacy of acute treatment with mGlu5 receptor antagonists on synaptic plasticity disruption by acute Aß (Hu et al., 2014; Jaeschke et al., 2015; Lindemann et al., 2015; Rammes et al., 2011) and are consistent with reports that repeated administration of these agents in APP transgenic mice ameliorates deficits in the performance

of learning tasks (Hamilton et al., 2016; Um et al., 2013). In addition to blocking over-activation of these receptors that has been triggered by excessive extracellular glutamate levels, selective antagonists can also prevent mGlu5 receptors being inappropriately activated by Aß-bound PrP, which, in conjunction with mGlu5, is a major extracellular toxic receptor for certain Aß oligomers (Beraldo et al., 2016; Haas et al., 2016; Hamilton et al., 2015; Lauren et al., 2009; Um et al., 2013). Hence, we examined the action of ICSM35, an antibody that prevents Aß oligomer binding to residues 95-110 of cellular PrP, and found that it was as effective as the mGlu5 receptor antagonists when administered repeatedly over a similar period of 3 days. This time frame is consistent with our previous finding that 3-day treatment with agents that either lower or bind Aß was sufficient to abrogate the impairment of LTP in the transgenic rat model (Qi et al., 2014). The finding of age-dependent accumulation of Aß oligomers in the brains of these rats supports previous observations of the presence of Aß oligomers intracellularly (Iulita et al., 2014; Leon et al., 2010). The levels we detected in younger rats were near the LLOQ, consistent with our previous research which failed to detect Aß oligomers using less sensitive Western blotting techniques (Qi et al., 2014). Despite the relatively low levels of detectable oligomers in the brains of younger rats, the ability of ICSM35 to reverse the LTP deficit strongly indicates that sufficient amounts are present extracellularly to bind PrP, which is an extracellular receptor for synaptotoxic Aß aggregates.

Some differences emerged between the acute effect of the different treatments in the two Aß models. Thus, acute basimglurant was effective in reversing the disruption of synaptic plasticity by injection of synthetic Aß 7 days previously, whereas a single administration of basimglurant either 2 or 16 h prior to HFS failed to reinstate LTP induction in the transgenic rats. On the other hand acute OXA/rGOT failed to reverse the LTP deficit in either the delayed Aß model or the transgenic rats. In the case of the persistent inhibition of LTP by

exogenous Aß, it seems likely that the deleterious effect is due to continued presence of the injected ADDL oligomers in the brain. It is known that Aß concentration remains elevated for at least 7 d after i.c.v. injection of ADDLs (Figueiredo et al., 2013; Zhang et al., 2014) and we found that injection of an anti-Aß antibody 2 h prior to HFS on day 7 completely reversed the LTP deficit (Ondrejcak et al., 2016). It seems unlikely that major structural changes mediate the disruption of LTP since baseline synaptic transmission and short-term plasticity were not significantly impaired on day 7 and the ability to induce LTP had recovered by day 14. Nevertheless we cannot rule out the possibility that a synaptotoxic conformation of Aß is stabilized in vivo or that structural changes accompany the functional deficit. In the case of the impairment of LTP in the transgenic animals something between 16 h and 3 days after initiating basimglurant treatment was required for full recovery. This could be taken to imply that structural changes may mediate the disruption of plasticity, though again, we failed to detect interference with baseline AMPA receptor-mediated synaptic transmission or short-term plasticity in the transgenic rats at 5-6 months of age (Qi et al., 2014). Consistent with these findings, Martino Adami et al (2017) reported that hippocampal CA1 structural integrity, as measured by synaptic density using electron microscopy or PSD-95 using western blots, is normal at 6 months. However, at 5-6 months of age both CA1 synaptic mitochondrial bioenergetics (Martino Adami et al., 2017) and NMDA receptor-mediated transmission (Qi et al., 2014) are reduced. Future studies should examine the possibility that the time course of recovery triggered by basimglurant corresponds to the time needed to restore normal synaptic bioenergetics, distribution of NMDA receptors or possibly synaptic structure.

By carrying out longitudinal studies, in addition to the direct comparison with control treatment groups, it was possible to track the time-course of reversal of the beneficial effects of the interventions in individual TG rats. In most groups the treatment effect had fully

reversed within one week of ceasing treatment, similar to the anti-Aß strategies investigated previously in this rat model using an anti-Aß antibody or secretase inhibitors (Qi et al., 2014). However, the rate of reversal of the beneficial effect of the anti-PrP antibody appeared to be somewhat slower than that present in animals treated with either the mGlu5 receptor antagonists or the blood-based glutamate scavengers. We have no explanation for this apparent difference.

Many different Aß cellular mechanisms have been reported to mediate Aß-induced increases in extracellular brain glutamate levels and synaptic plasticity disruption (Viola and Klein, 2015; Zadori et al., 2014). Currently it is unknown if Aß-induced increases in extracellular glutamate concentration are at least partly mediated by binding to cellular PrP. Since activation of the PrP co-receptor mGlu5 receptors on astrocytes can trigger non-vesicular glutamate release (Angulo et al., 2004; D'Ascenzo et al., 2007; Fellin et al., 2004) it is possible that Aß binding to PrP on astrocytes may increase glutamate release. Interestingly mGlu5 receptor triggered glutamate release from astrocytes appears to preferentially access extrasynaptic GluN2B-containing NMDA receptors (Angulo et al., 2004; D'Ascenzo et al., 2007; Fellin et al., 2004), and these receptors have been strongly implicated in mediating the acute inhibition of LTP by Aß oligomers (Hu et al., 2008; Li et al., 2009). Moreover, Aß oligomer-induced glutamate release from astrocytes has been reported to increase extrasynaptic NMDA receptor activation that may contribute to synaptic damage (Talantova et al., 2013).

Consistent with our findings from previous studies of acute i.v. treatment with oxaloacetate alone or in combination with rGOT in anaesthetized rats (Zhang et al., 2016) we now report that repeated administration of rGOT combined with acute i.v. treatment with these agents also did not to disrupt control LTP in either anaesthetized or awake freely behaving animals. The lack of detectible deleterious effects of repeated treatment with these

blood-based glutamate scavengers on control LTP presumably reflects, as discussed above, their relative selective ability to reduce excessive, non-physiological, levels of brain glutamate (Castillo et al., 2016). The lack of significant effect on LTP in control animals indicates that potentially there is a beneficial dose range of the agents against Aß that does not disrupt physiological plasticity. Further study will be required to rule out the possibility that other synaptic functions and plasticity induced by different protocols in other pathways are not affected by repeated treatment with blood-based glutamate scavengers. Significantly, the blood concentration of endogenous GOT correlates positively with stroke outcome and is known to reach relatively high levels in healthy humans (Campos et al., 2011a; Castillo et al., 2016; Tian et al., 2012).

Similarly, the repeated dosing schedule for the mGlu5 receptor antagonists used here did not affect control LTP at 3 h post-HFS. However, it seems probable that later time points or other forms of plasticity may be impeded in the dose range that ameliorated the detrimental effects of Aß. Extensive research has shown that certain forms of LTP, LTD and depotentiation are inhibited by these agents in brain areas including the hippocampus under physiological conditions (Anwyl, 2009; Buschler and Manahan-Vaughan, 2016; Collingridge et al., 2010; Qi et al., 2013; Zarnadze et al., 2016) . Indeed, mGlu5 receptor activation can "reinforce" the induction of longer-lasting forms of LTP, and late-phase LTP may be more sensitive than early phase LTP to block of group I receptors (Balschun et al., 1999; Manahan-Vaughan, 1997). Somewhat similarly, behavioral effects of blocking these receptors in animals appear to be relatively task specific (Ahnaou et al., 2015). Indeed, doses of mGlu5 receptor antagonists that were effective in attenuating behavioural deficits in APP transgenic mouse models also slightly interfered with performance in non-TG mice (Hamilton et al., 2014; Um et al., 2013). Thus on the one hand, there is a generally accepted view that ongoing activation of mGlu5 receptors is likely to be necessary for normal cognitive function and

underlying plasticity mechanisms. On the other hand, in clinical trials of antagonists, including basimglurant, at potentially therapeutic doses, dizziness and visual hallucinations rather than cognitive impairment have been found to be dose-limiting in humans (Berry-Kravis et al., 2016; Reilmann et al., 2015; Scharf et al., 2015; Tison et al., 2016).

Further pre-clinical studies of rGOT treatment that extend our present findings will be required to determine if such an approach may prove promising as a potential therapeutic in very early stages of the disease. In the case of mGlu5 receptor antagonists that have already been used safely in human studies the current evidence indicates that adverse effects may not markedly limit their potential benefits. A similar case can be made for antibodies preventing Aß oligomer binding to PrP, although to date they have only been tested in preclinical models (Klyubin et al., 2014a). Certainly, a strategy of targeting both glutamate clearance and mGlu5 receptors/PrP mechanisms warrants further investigation to determine if such a dual approach can limit potential adverse effects while maintaining efficacy.

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Competing financial interests

John Collinge is a director and shareholder of D-Gen Limited (London), an academic spin-out company in the field of prion diagnosis, decontamination and therapeutics. D-Gen

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Fig. 1. A single injection of ADDLs persistently inhibits LTP in the CA1 area of the rat hippocampus *in vivo*. (*A*) In control animals, that were injected i.c.v. with vehicle (Veh) under recovery anaesthesia and recorded from 7-14 d later under non-recovery anaesthesia, the application of HFS (arrow) induced robust LTP that lasted at least 3 h ($p<0.05$ compared with pre-HFS baseline, paired *t*-test). In contrast, in animals injected 7-9 days previously with an ADDL preparation of Aß1-42 enriched in protofibrils (Aß7, 480 pmol, i.c.v.), a decremental LTP was induced ($p>0.05$ compared with pre-HFS baseline at 3h post-HFS). Interestingly, the inhibition of LTP by Aß was no longer detectible 14 d after the injection (Aß14). Insets show representative EPSP traces at the times indicated. Calibration bars: Vertical, 1.0 mV; horizontal, 10 ms. (*B*) Summary bar chart comparing the magnitude of synaptic potentiation between treatment groups at 3 h post-HFS. Values are the mean ± SEM fEPSP amplitude expressed as a percentage of the pre-HFS baseline. (*C, D*) Lack of change in either (*C*) the input/output relationship as measured by the magnitude of the response to different intensities of stimulation (50, 75 and 100% maximum) or (*D*) short term plasticity as measured by the magnitude of the paired-pulse facilitation ratio at a 40 ms inter pulse interval. *$p<0.05$, one-way ANOVA followed by Bonferroni test.

Fig. 2. Acute systemic treatment with the blood-based glutamate scavenger oxaloacetate alone or in combination with recombinant glutamate oxaloacetate transaminase failed to reverse synthetic Aß-mediated persistent inhibition of LTP. (*A*) In control animals, that were injected i.c.v. with vehicle for Aß and seven days later injected i.v. ('plus' symbol) with vehicle for oxaloacetate (Veh+Veh), the application of 3 sets of HFS (3 arrows) induced robust LTP that was relatively stable for at least 3 h ($p<0.05$ compared with pre-HFS baseline, paired *t*-test). In contrast, in animals injected with an ADDL preparation of Aß1-42 enriched in protofibrils (Aß, i.c.v.) 7 days before i.v. vehicle (Aß+Veh), a decremental LTP was induced by the same HFS protocol ($p>0.05$ compared with pre-HFS baseline at 3h post-

HFS). (*B*) In animals that received oxaloacetate i.v. (plus symbol, 35 mg/kg in 1 ml) 1 h prior to HFS (arrows) on day 7 post-Aß administration, the application of HFS failed to induce stable LTP (Aß+OXA) (p>0.05 compared with pre-HFS baseline at 3 h post-HFS and Aß+Veh group). (*C*) We tested the effect of a dose (0.39 mg/rat i.v.) of recombinant type 1 GOT (rGOT) that when administered alone did not prevent the inhibition of LTP by Aß (rGOT/Veh+Aß) (p>0.05 compared with pre-HFS baseline at 3 h post-HFS), together with oxaloacetate (rGOT/OXA+Aß). However, no significant beneficial effect was observed (p>0.05 compared with pre-HFS baseline at 3 h post-HFS). (*D*, *E*) Summary bar chart comparing the magnitude of synaptic potentiation between treatment groups at 3 h post-HFS. Values are the mean ± SEM fEPSP amplitude expressed as a percentage of the pre-HFS baseline. *p<0.05, unpaired *t*-test or one-way ANOVA followed by Bonferroni test.

Fig. 3. Repeated treatment with rGOT alone or in combination with acute treatment of oxaloacetate reverses synthetic Aß-mediated persistent inhibition of LTP. (*A*) HFS (arrows) induced robust LTP in control animals that received vehicle injections (i.c.v. on day 0; 0.2 ml/day, s.c. on days 0-6; and 1 ml i.v. on the day of recording, plus symbol) (Veh+Veh+Veh) (p<0.05 compared with pre-HFS baseline). In contrast, LTP was inhibited seven days after i.c.v. injection of Aß (Aß+Veh+Veh) (p>0.05 compared with pre-HFS baseline, p<0.05 compared with Veh+Veh+Veh group). (*B*) In Aß pre-treated rats followed by repeated rGOT alone (s.c. + i.v.) (Aß+rGOT+Veh/rGOT), or in combination with oxaloacetate on day 7 (Aß+rGOT+OXA/rGOT), application of HFS induced LTP that was indistinguishable from vehicle control group (p>0.05 compared with Veh+Veh+Veh group, p<0.05 compared with Aß+Veh+Veh group). (*C*) Summary bar chart comparing the magnitude of synaptic potentiation at 3 h post-HFS between treatment groups. Values are the mean ± SEM fEPSP amplitude expressed as a percentage of the pre-HFS baseline. (*D*) Compared with vehicle control group, the serum glutamate levels were significantly reduced in both

Aß+rGOT+Veh/rGOT (n=5) and Aß+rGOT+OXA/rGOT (n=5) groups. *p<0.05, one-way ANOVA followed by Bonferroni test.

Fig. 4. Both acute and repeated treatment with the mGlu5 receptor antagonist basimglurant abrogates synthetic Aß-mediated persistent inhibition of LTP. (*A*) Whereas LTP was inhibited in Aß (i.c.v.) pre-injected animals, in rats given basimglurant (2 mg/kg i.p., single injection) (Aß+BMG) HFS now induced a robust LTP (p<0.05 compared with pre-HFS baseline, paired *t*-test). (*B*) Similarly, in rats given basimglurant (3 daily injections of 2 mg/kg i.p.) (Aß+BMG) the application of HFS 2 h after the last injection induced robust LTP (p<0.05). (*C*) In control rats that received vehicle (0.3ml, once a day for 3 days) (Veh), the application of HFS, 2 h after the last injection, induced robust LTP that was relatively stable for at least 3 h (p<0.05). HFS induced similar LTP in animals treated with basimglurant (2 mg/kg/day for 3 days) in the same manner (p<0.05). (*D-F*) Summary bar charts comparing the magnitude of synaptic potentiation at 3 h post-HFS between treatment groups. Values are the mean ± SEM fEPSP amplitude expressed as a percentage of the pre-HFS baseline. *p<0.05, unpaired *t*-test.

Fig. 5. Acute co-treatment with the blood-based glutamate scavengers oxaloacetate and rGOT fails to reverse the inhibition of LTP in freely behaving APP over-expressing transgenic rats. (*A*) An oligomer-specific DELFIA reveals an age-dependent accumulation of Aβ assemblies in the brain of McGill-R-Thy1-APP TG rats. Soluble extracts of rat brains were assayed by DELFIA using the anti-Aβ oligomer selective monoclonal antibody 1C22. The lower limit of quantification (LLOQ, dotted line) was 80 pM. Oligomers were detected in 11 of 28 TG rats aged 3-13 months but in none of the 10 WT littermates of a similar age range. Values are the mean±SD for each rat. (*B*) A single set of HFS (arrow) failed to induce LTP in APP TG animals (TG) (p>0.05 compared with pre-HFS baseline), while this HFS protocol induced robust LTP in WT littermates (WT) (p<0.05 compared with pre-HFS

baseline, $p<0.05$ compared with TG rats). (*C*) In TG animals that received oxaloacetate and rGOT (35mg/kg and 0.39mg/rat respectively, i.v.), application of HFS 1 h later failed to induce LTP ($p>0.05$ compared with pre-HFS baseline). This treatment did not appear to affect control LTP in WT littermates ($p<0.05$ compared with pre-HFS baseline, $p<0.05$ compared with TG rats). (*D,E*) Summary bar chart comparing the magnitude of synaptic potentiation at 3 h post-HFS between treatment groups. Values are the mean ± SEM fEPSP amplitude expressed as a percentage of the pre-HFS baseline. *$p<0.05$, unpaired *t*-test.

Fig. 6. Repeated treatment of rGOT combined with acute treatment of rGOT and oxaloacetate reverse the LTP deficit in APP TG rats. (*A*) In TG rats, HFS (arrow) failed to induce robust LTP in animals that received repeated s.c. (0.2 ml/day, not illustrated) and i.v. (1 ml, not illustrated) injection of proper vehicle (Veh+Veh) ($p>0.05$ compared with pre-HFS baseline). Repeated s.c. injection of rGOT (0.39 mg/rat) combined with oxaloacetate (35 mg/kg, i.v.) and rGOT (0.39 mg/rat, i.v.) pre-treated 1 h prior to HFS, LTP was reversed (rGOT+rGOT/OXA) ($p<0.05$ compared with pre-HFS baseline, $p<0.05$ compared with Veh+Veh group). (*B*) Summary bar chart comparing the magnitude of synaptic potentiation at 3 h post-HFS between treatment groups in (*A*). (*C, D*) The recovery from the impairment in synaptic plasticity lasted for less than a week in the drug-treated group. The same HFS protocol applied either just before (pre) or one week after (post) the injections of drugs failed to induce LTP. Data for individual animals are shown in (*C*) and summarized statistically in the bar charts (*D*). (*E*) In WT litter mates of Tg animals, either vehicle (Veh+Veh) or drug (rGOT+rGOT/OXA) treatments have significant effect on LTP ($p<0.05$ compared with pre-HFS baseline, $p>0.05$ compared with Veh+Veh group). (*F*) Summary bar chart comparing the magnitude of synaptic potentiation at 3 h post-HFS between treatment groups in (*E*). (*G, H*) The same HFS protocol applied either just before (pre) or one week after (post) the injections of drugs n=6 induced LTP that have no significant difference. Data for individual

animals are shown in (G) and summarized statistically in the bar charts (*H*). Values are the mean ± SEM fEPSP amplitude expressed as a percentage of the pre-HFS baseline. *p<0.05, unpaired *t*-test or one-way ANOVA followed by Bonferroni test.

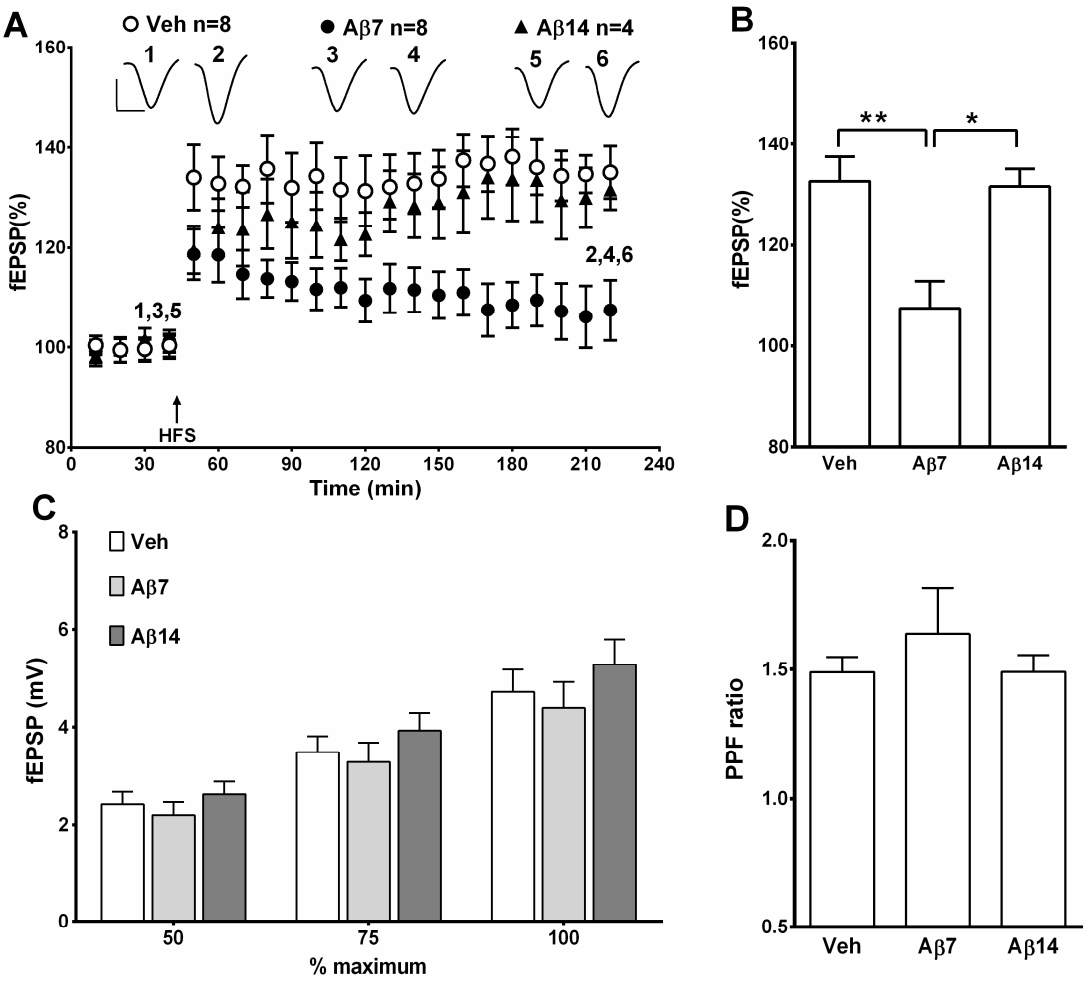
Fig. 7. Acute treatment with the mGlu5 receptor antagonist basimglurant failed to reverse the LTP deficit in APP TG rats. (*A*) In TG rats HFS failed to induce LTP 2 h after being treated with basimglurant (BMG, n=5, 2 mg/kg, i.p.), similar to 7 days before treatment (Pre). (*D*) Similarly, HFS failed to induce LTP 16 h after being treated with basimglurant (BMG, n=5, 3 mg/kg, i.p.). Magnitude of LTP (*B,E*) measured 3 h post-HFS tracked in individual rats or (*C,F*) summarized statistically in a bar chart. Values are the mean ± S.E.M. % pre-HFS baseline EPSP amplitude.

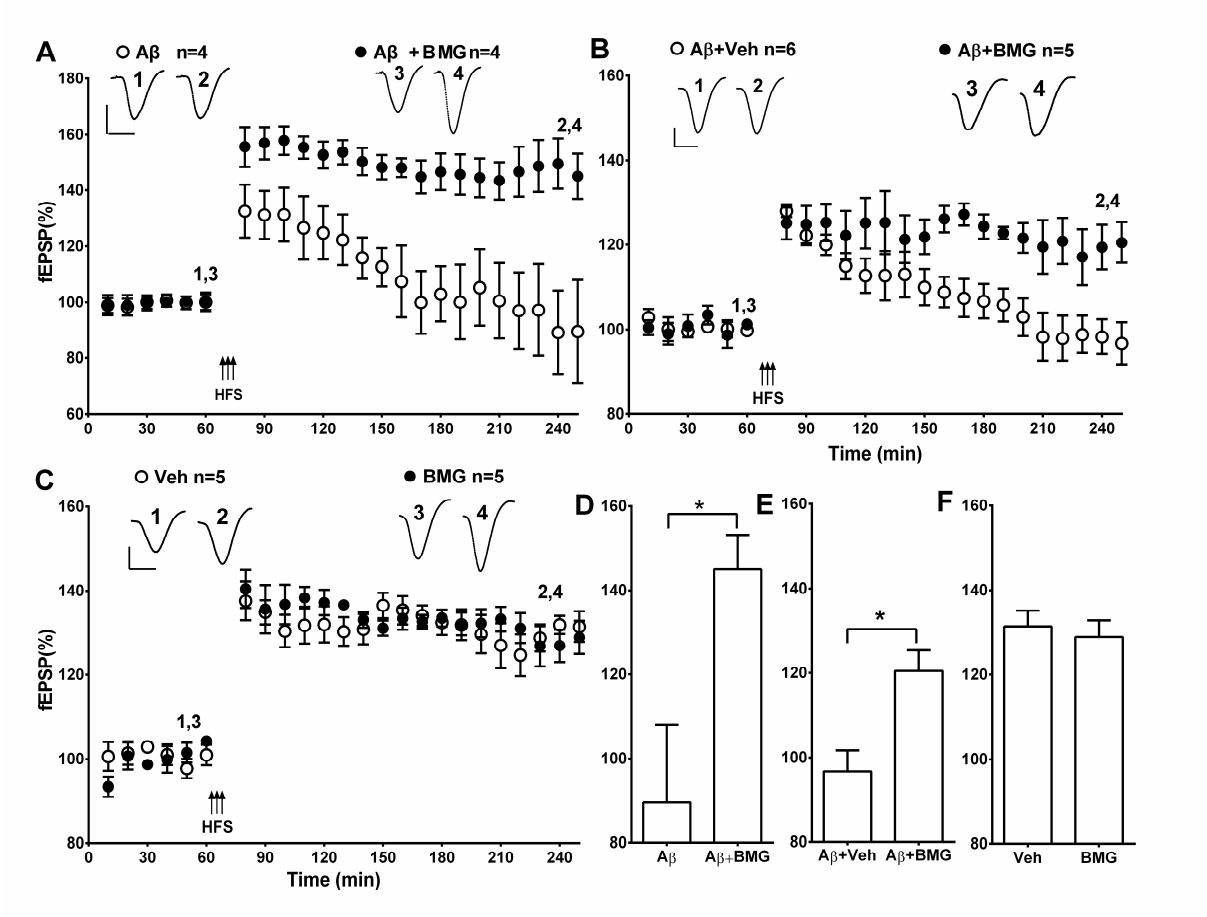
Fig. 8. Repeated treatment with the mGlu5 receptor antagonist basimglurant transiently reversed the LTP deficit in APP TG rats. (*A*) In TG rats HFS triggered robust and stable LTP while being treated with basimglurant (n=6, 2 mg/kg/day for 3 days, i.p.) but not before (pre) or 7 days after (post) treatment. (*B*) Magnitude of LTP measured 3 h post-HFS tracked in individual rats (B) or summarized statistically in the bar chart (*C*). *p<0.05 compared with pretreatment levels of potentiation in the same animals. Values are the mean ± S.E.M. % pre-HFS baseline EPSP amplitude. *p<0.05, one-way ANOVA followed by Bonferroni test.

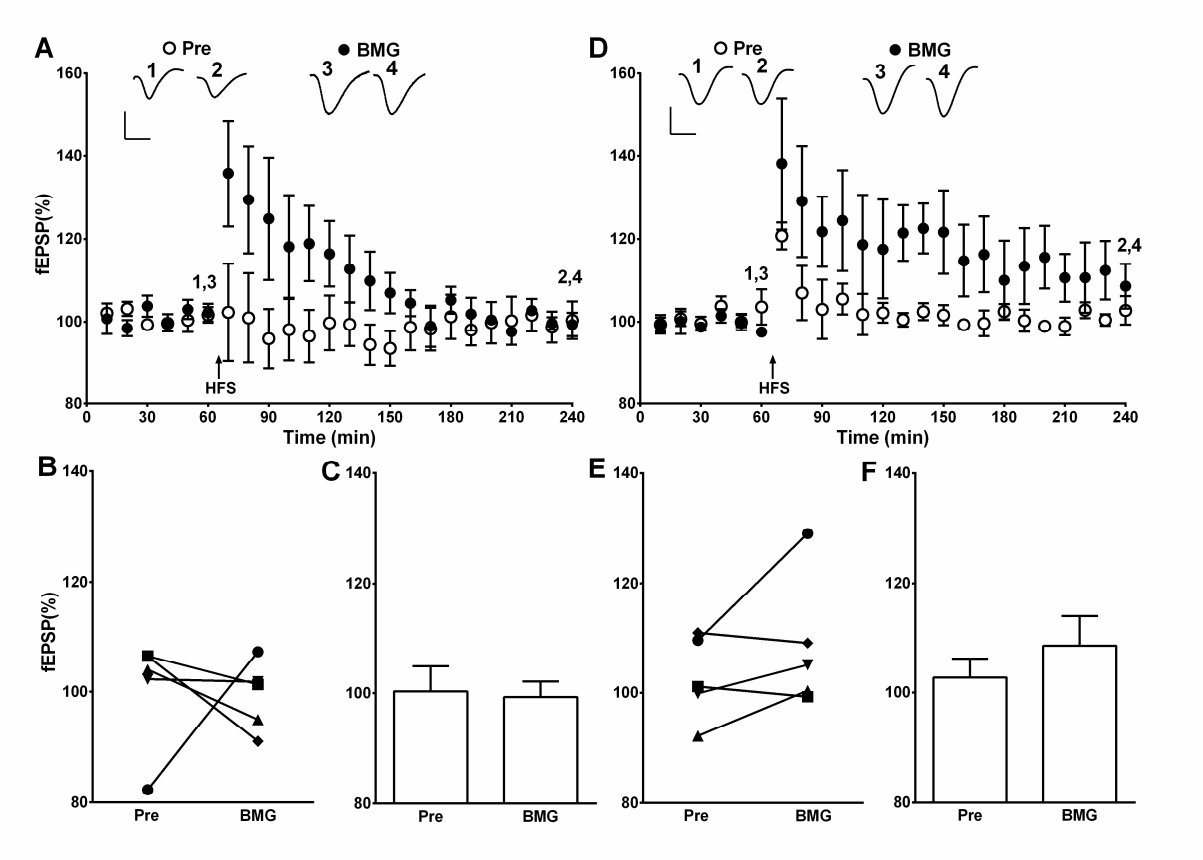
Fig. 9. Repeated treatment with the mGlu5 receptor antagonist CTEP transiently reversed the LTP deficit in APP TG rats. (*A*) In TG rats that received vehicle (0.3 ml, twice over 3 days) (Veh), the application of HFS, 2 h after the last injection, failed to induce robust LTP (p>0.05 compared with pre-HFS baseline, paired *t*-test). However, HFS induced robust LTP that was relatively stable for at least 3 h in animals treated with CTEP (2 mg/kg, twice in 3 days) (p<0.05 compared with Veh). (*B*) Summary bar chart comparing the magnitude of synaptic potentiation at 3 h post-HFS between treatment groups in (*A*). (*C, D*) The recovery from the

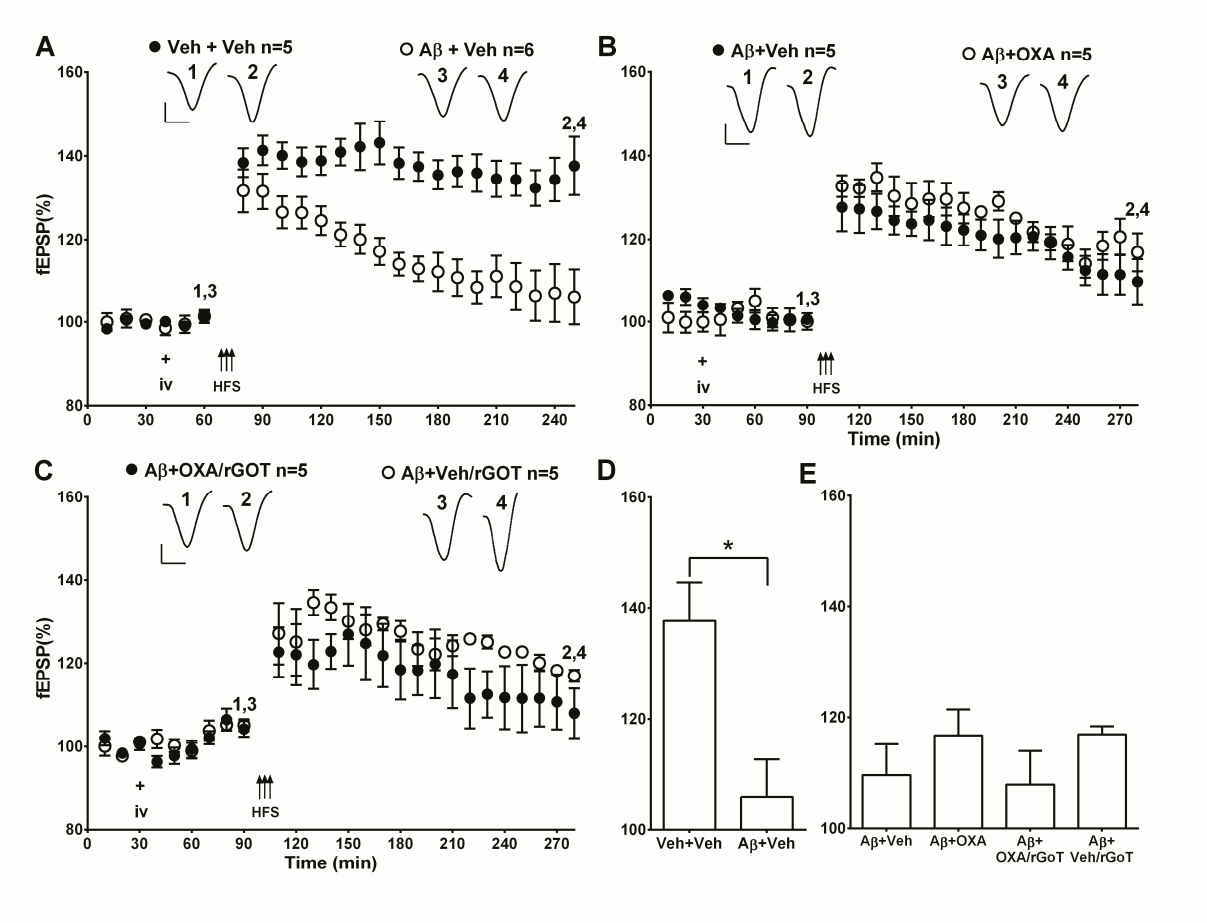
impairment in synaptic plasticity lasted for less than a week in the drug-treated group. The same HFS protocol applied either just before (pre) or one week after (post) the injections of drugs failed to induce LTP. Data for individual animals are shown in (*C*) and summarized statistically in the bar charts (*D*). *p<0.05, unpaired *t*-test or one-way ANOVA followed by Bonferroni test.

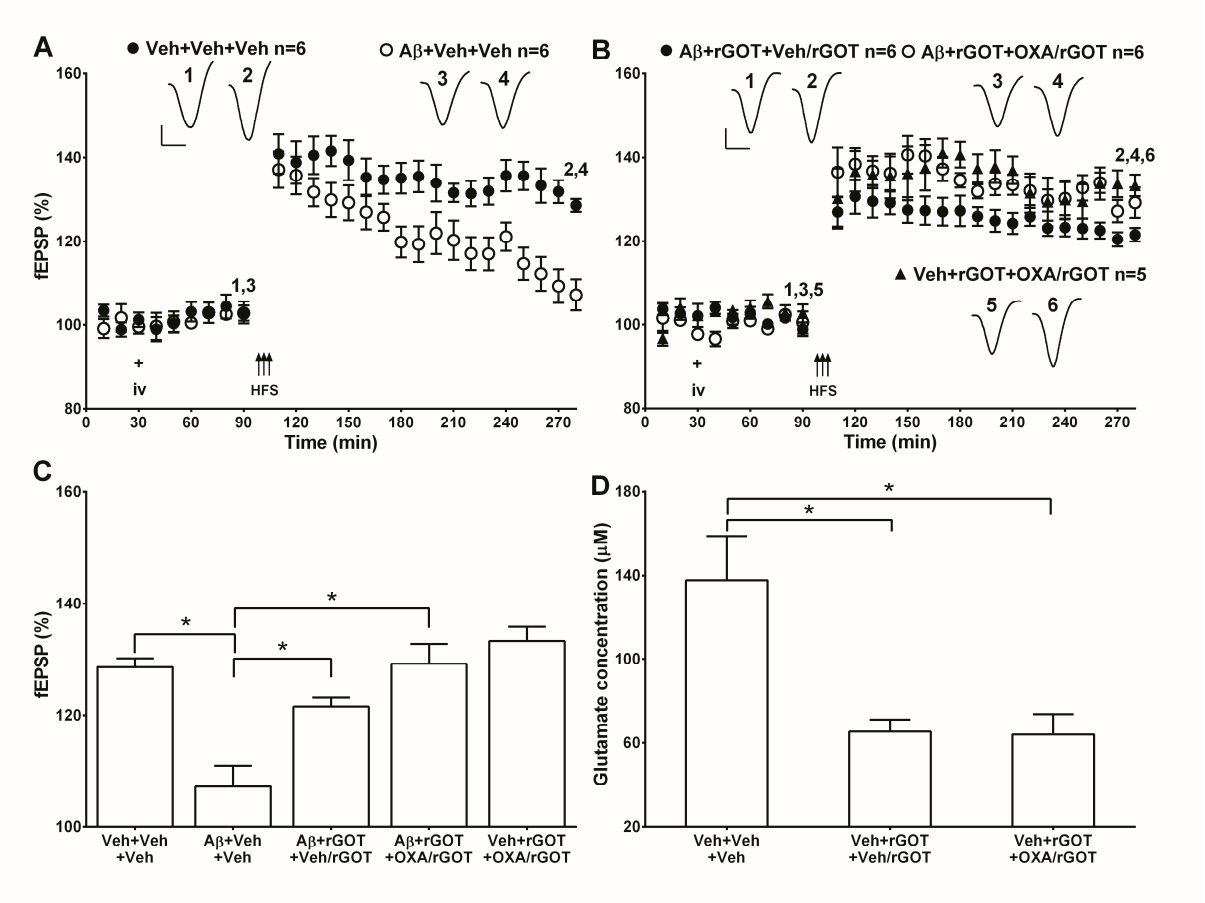
Fig. 10. Repeated treatment with an antibody that blocks Aß oligomer binding to cellular prion protein, ICSM35, transiently reversed the LTP deficit in APP TG rats. (*A*) Three-day treatment with ICSM35 (5 X 60 µg injections over 3 days, i.c.v.) transiently restored the ability to induce LTP in TG rats. In animals treated with ICSM35, but not with an isotype control IgG antibody BRIC126, HFS triggered robust LTP. Insets show representative EPSP traces at the times indicated. Calibration bars: Vertical, 1.0 mV; horizontal, 10 ms. (*B*) Summary bar chart of the magnitude of synaptic potentiation at 3 h post-HFS. *p<0.05, unpaired *t*-test. (*C*) Data for individual animals are shown for before (pre), during and 2 weeks after ICSM35. Data from *C* summarized statistically in bar charts. *P<0.05 compared with pre. Values are the mean ± S.E.M. % pre-HFS baseline EPSP amplitude. *p<0.05, one-way ANOVA followed by Bonferroni test.

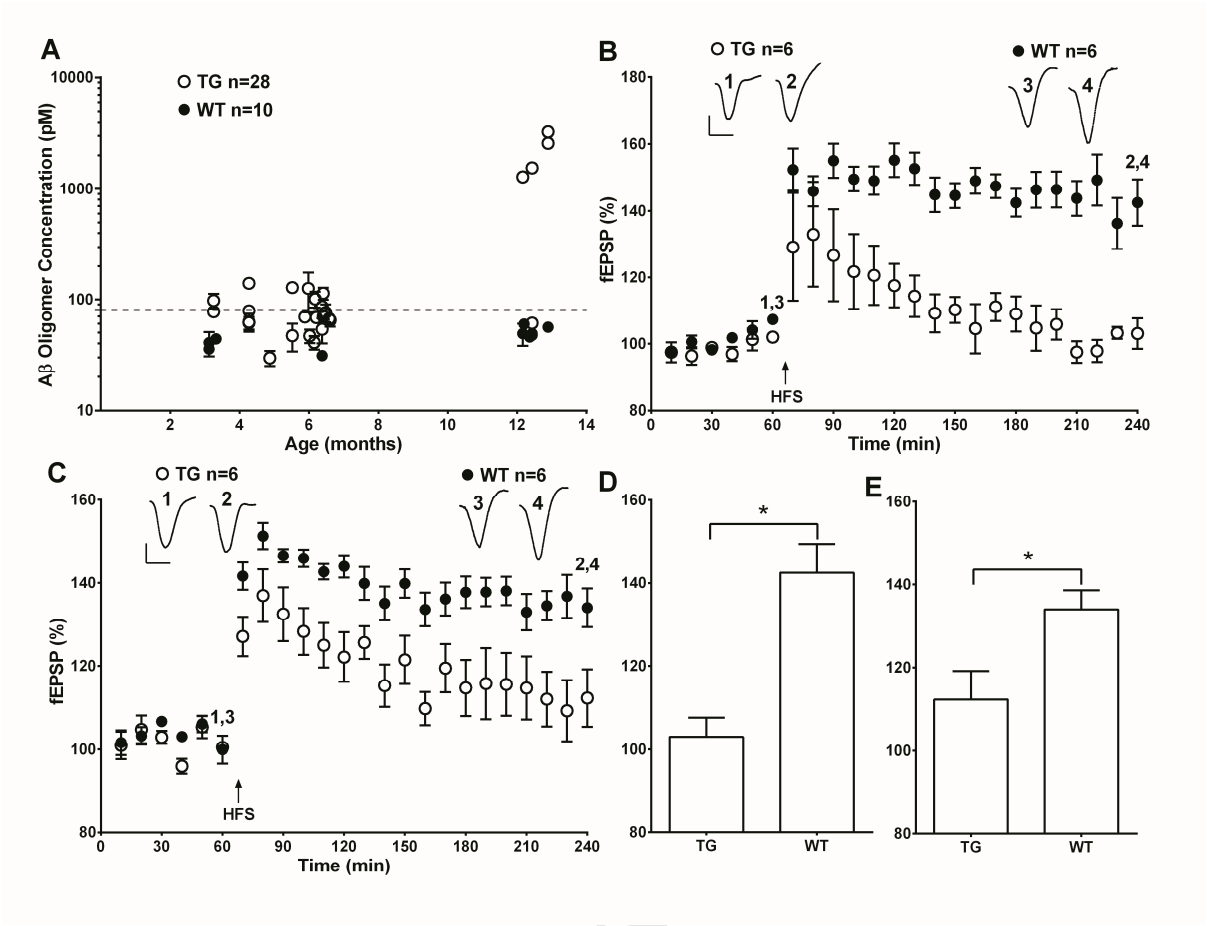


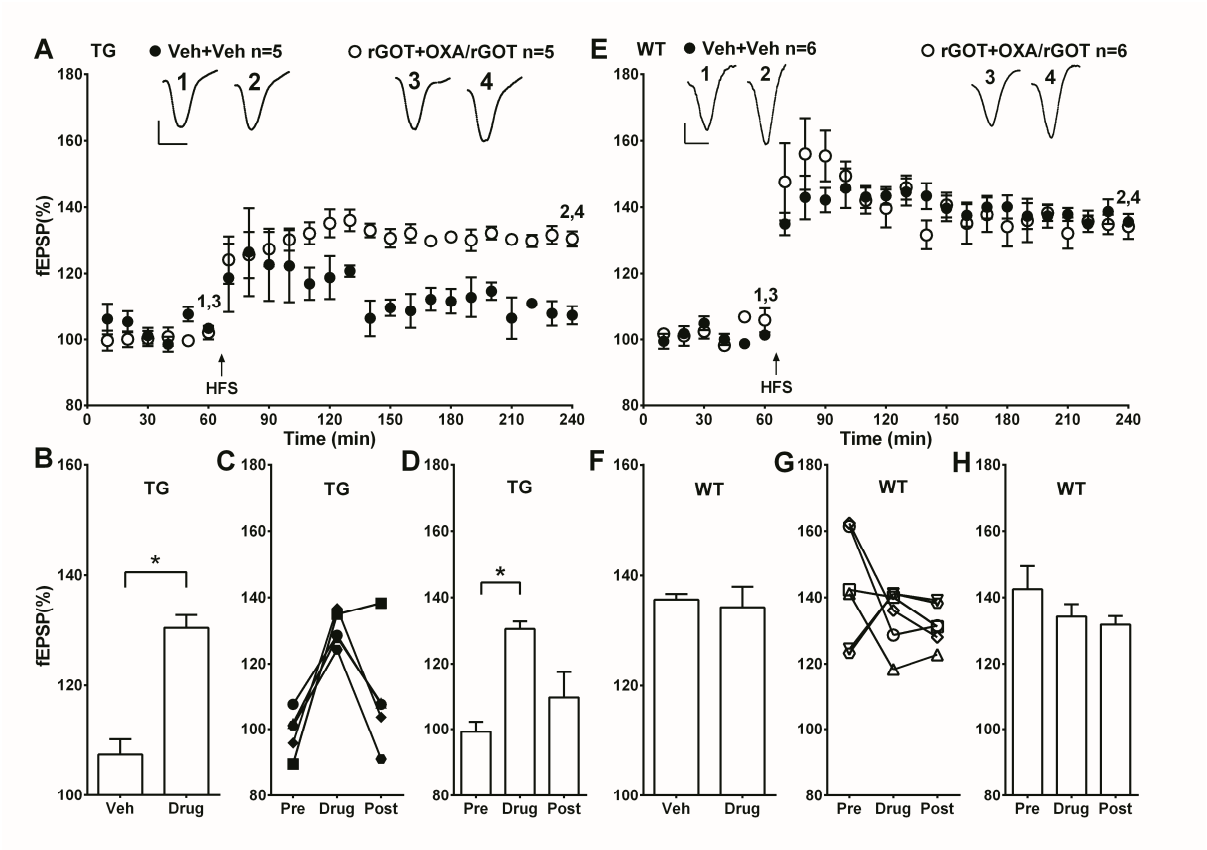


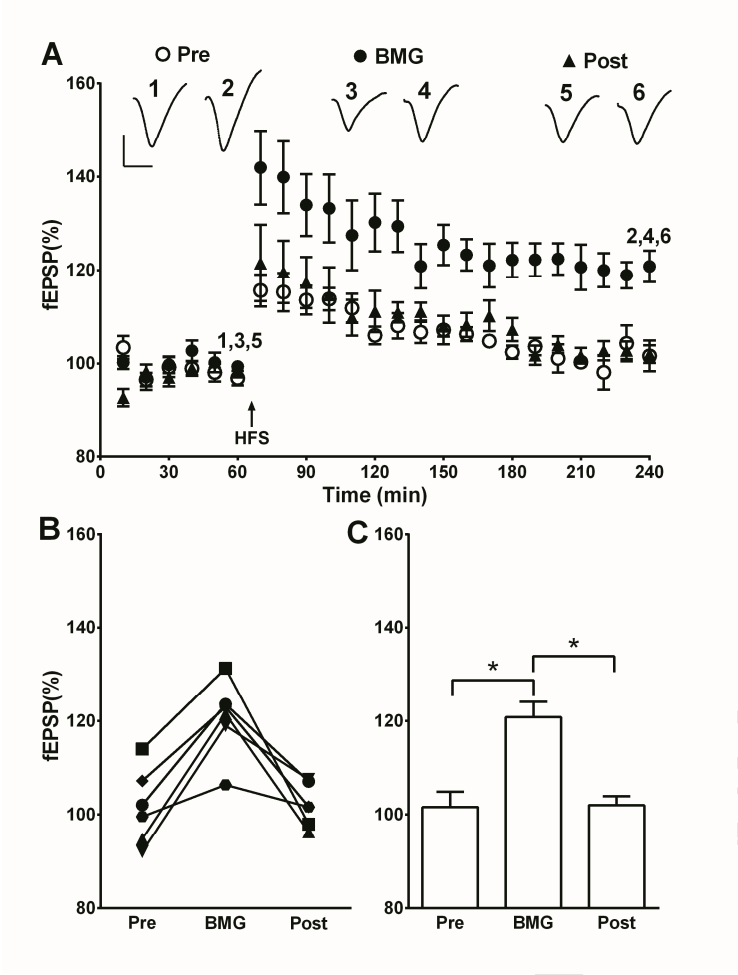


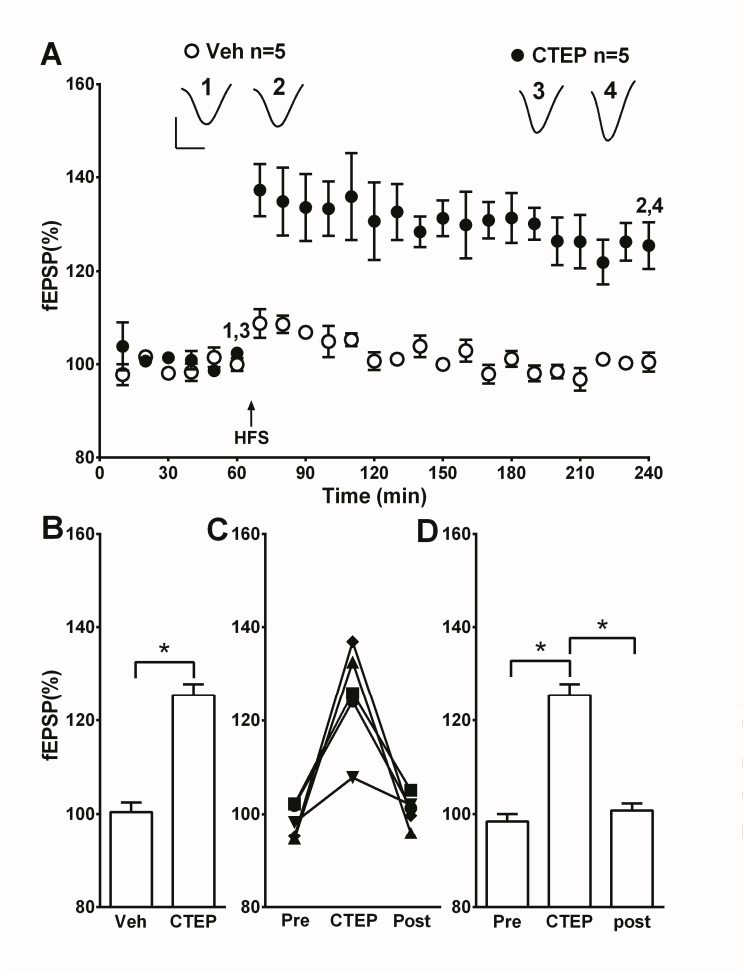


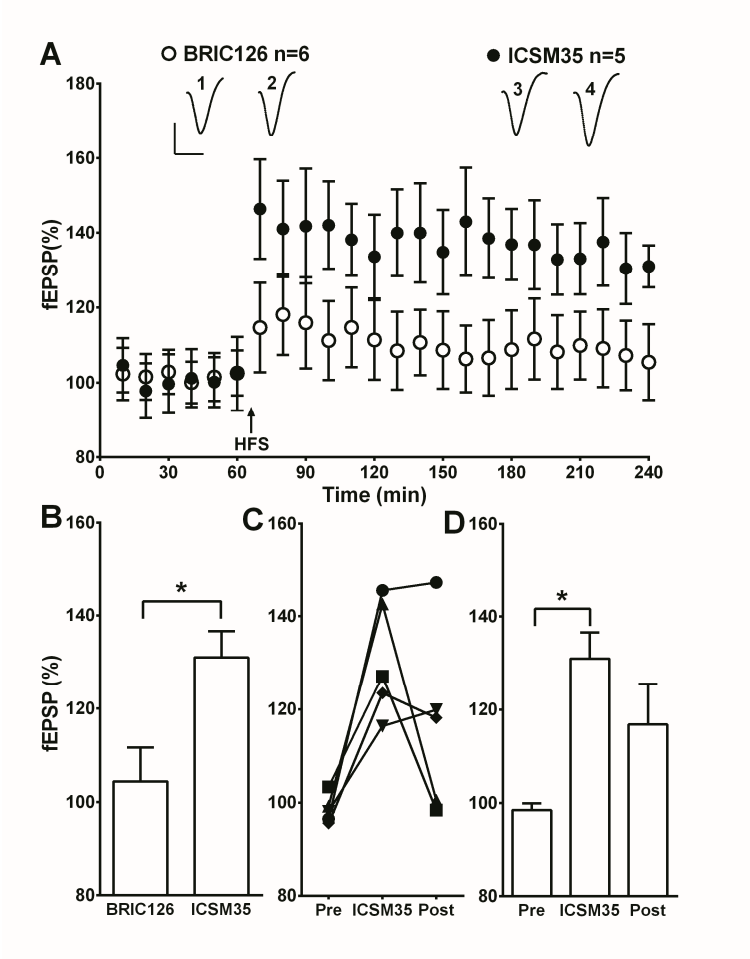












Highlights

A blood-based glutamate scavenger reverses the persistent disruption of synaptic plasticity by Aß oligomers

An mGlu5R antagonist or antibody that prevents Aß oligomer binding to PrP, act similarly

Longitudinal studies in freely behaving transgenic rats reveal the rapid time-course