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ORIGINAL ARTICLE

Proneurogenic Group II mGluR antagonist improves learning and reduces anxiety in Alzheimer Aβ oligomer mouse

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Proneurogenic compounds have recently shown promise in some mouse models of Alzheimer's pathology. Antagonists at Group II metabotropic glutamate receptors (Group II mGluR: $mGlu_2$, $mGlu_3$) are reported to stimulate neurogenesis. Agonists at those receptors trigger γ-secretase-inhibitor-sensitive biogenesis of Aβ42 peptides from isolated synaptic terminals, which is selectively suppressed by antagonist pretreatment. We have assessed the therapeutic potential of chronic pharmacological inhibition of Group II mGluR in Dutch APP (Alzheimer's amyloid precursor protein E693Q) transgenic mice that accumulate Dutch amyloid-β (Aβ) oligomers but never develop Aβ plaques. BCI-838 is a clinically well-tolerated, orally bioavailable, investigational prodrug that delivers to the brain BCI-632, the active Group II mGluR antagonist metabolite. Dutch Aβ-oligomer-forming APP transgenic mice (APP E693Q) were dosed with BCI-838 for 3 months. Chronic treatment with BCI-838 was associated with reversal of transgene-related amnestic behavior, reduction in anxiety, reduction in levels of brain Aβ monomers and oligomers, and stimulation of hippocampal neurogenesis. Group II mGluR inhibition may offer a unique package of relevant properties as an Alzheimer's disease therapeutic or prophylactic by providing both attenuation of neuropathology and stimulation of repair.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to dementia and neuropsychological symptoms such as anxiety and depression.[1] Currently, no cure or disease-modifying treatment is available. Cholinesterase inhibitors (donepezil, rivastigmine and galantamine) and an extrasynaptic NMDA receptor antagonist (memantine) are approved for the treatment of AD, but these offer only temporary symptomatic benefit and even that response occurs in only a subset of patients.[2]

Synaptic dysfunction in AD begins insidiously during the preclinical stage of the disease.[3,4] One of the suspected causes of this dysfunction is an accumulation of neurotoxic oligomers of the amyloid-β peptide (oligomeric Aβ (oAβ)),[5] formation of which is dependent on the concentration of highly aggregatable Aβ42 peptides.[6] We previously discovered that stimulation of Group II metabotropic glutamate receptors (Group II mGluR: $mGlu_2$, $mGlu_3$) triggers selective production and release of Aβ42 peptides from isolated intact nerve terminals, while having little effect on the release of the less aggregatable Aβ40 peptides. This neurotransmission-induced shift in the ratio of Aβ42:Aβ40

peptides is a potentially amyloidogenic synaptic event that can be selectively suppressed by Group II mGluR antagonist pretreatment.[7] We thus hypothesized that chronic suppression of Group II mGluR signaling may slow disease progression by reducing the accumulation of Aβ oligomers. When considering this strategy in the context of the human illness, it is worth noting that one of the Group II mGluR subtypes, $mGlu_2$, is expressed at abnormally elevated levels in the AD hippocampus,[8] suggesting that, in human sporadic AD, overactivation of $mGlu_2$ may contribute to dysregulation of Aβ peptide production/speciation and/or release.

In an independent area of neuropharmacology research focusing on the possible antidepressant and anxiolytic effects of proneurogenic drugs, Group II mGluR antagonists have been demonstrated to enhance learning and memory behaviors and to alleviate depressive and anxiety behaviors in rodents.[9–12] These actions could be related to the ability of Group II mGluR antagonists to stimulate hippocampal neurogenesis.[13] Therefore, when the selective activity of Group II mGluR antagonists to block synaptic Aβ42 production *in vitro* is taken together with the

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various activities listed above, these compounds would appear to possess a collection of properties that may be relevant to the prevention or treatment of AD. It is worth noting that other investigators have recently demonstrated that proneurogenic compounds are beneficial in mouse models of neurodegeneration.[14–17] The notion of a compound that combines reduction of pathology, improvement in cognitive function, anxiolysis and stimulation of neurogenesis is of special interest in the current era of AD research, when the field, in general, is pivoting toward AD prevention,[1] leaving little in the way of a drug pipeline for the 35.6 million patients currently suffering from AD worldwide.

Dysfunctional neurogenesis has been reported in various AD transgenic mouse models[18–26] (reviewed in Lazarov and Marr[27]; Marlatt and Lucassen[28]; Mu and Gage[29]; and Winner et al.[30]). In commonly used triple transgenic AD mice carrying three mutant genes (amyloid precursor protein (APP), presenilin 1 (PS1) and tau), reduced proliferation was reported to be associated with the presence of Aβ plaques and the number of Aβ-containing neurons in the hippocampus.[22] A familial AD-linked PS1 mutation prevented the neurogenesis response typically evoked by environmental enrichment.[18] Wild-type neural stem cells cocultured with familial AD-mutant-PS1-expressing microglia showed reduced proliferation and neural lineage commitment, suggesting that soluble microglial releasate may contain a non-cell-autonomous factor that compromises adult hippocampal neurogenesis in vivo.[18] Silencing of mutant PS1 in excitatory neurons including dentate granule cells significantly rescued the proliferation and survival of hippocampal neural progenitor cells in familial AD-mutant-PS1-expressing mice in response to environment enrichment, again indicating the presence of one or more non-cell-autonomous factors for regulating progenitor cells.[31]

To achieve continuous pharmacological inhibition of Group II mGluR, a novel, orally bioavailable prodrug, BCI-838, was used to deliver its active metabolite, BCI-632, a potent Group II mGluR inhibitor, to the brain. Daily oral dosing of BCI-838 results in steady-state levels of BCI-632 in the brain, the effective concentration of which lasts for 22 h.[32] The Dutch APP transgenic mice (APP E693Q)[33] were chosen for this study because they provide a model of central nervous system accumulation of Dutch Aβ oligomers in the absence of Aβ fibrils. A substantial body of literature implicates the Aβ oligomer as the key neurotoxin in AD, and this mouse provides a model that enables the testing of that hypothesis selectively. Other models (for example, Tg2576, TgCRND8, Borchelt PS/APP and 3xTg) also accumulate Aβ oligomers, but those models also accumulate fibrils, confounding the ability to implicate a particular biophysical Aβ species. These Dutch APP mice accumulate cerebral amyloid angiopathy to a modest degree; other mice, such as Dutch-Iowa APP mice, develop much more robust cerebral amyloid angiopathy and therefore are more suitable for the assessment of cerebral amyloid angiopathy-reducing drugs.[33,34]

Here, we report the results of in vivo testing of the Group II mGluR antagonist in APP transgenic mice to assess its potential symptomatic and disease-modifying capabilities.

MATERIALS AND METHODS

Serial detergent fractionation with ultracentrifugation
Snap-frozen tissue was homogenized by 20 up-and-down strokes of a glass-Teflon homogenizer at 500 r.p.m. in ice-cold tris-buffered saline (TBS; pH 7.6) containing protease/phosphatase inhibitors (1 mM EDTA, 1 mM Na_3VO_4, 5 μM $ZnCl_2$, 100 mM NaF, 1 μM pepstatin, 1 mM PMSF, mini-complete protease inhibitor tablet (Roche, Indianapolis, IN, USA)). The TBS homogenate was then ultracentrifuged at 100 000 x g for 1 h at 4 °C and the supernatant was removed, aliquoted and stored at − 80 °C until analysis. The TBS-insoluble pellet was then homogenized in TBS (pH 7.6) containing protease/phosphatase inhibitors and 1% (v/v) Triton-

X-100 and ultracentrifuged as above. The supernatant was collected and the Triton-X-100-insoluble pellet was then homogenized in 70% formic acid, ultracentrifuged as above, and neutralized by 1:20 dilution into 1 M Tris (pH 11.0). All homogenization and sample collection steps were performed on ice and samples were aliquoted to avoid denaturing of protein by repeated freeze–thaw cycles.

Aβ assays
Both dot blot immunobinding[35] and enzyme-linked immunosorbent assays are accepted in the field for the measurement of levels of oAβ in tissue extracts. For analysis of native oAβ protein structure, 2–4 μl native protein samples from each fraction were spotted onto activated/pre-wetted polyvinylidene difluoride membrane (0.22 μm; Millipore, Billerica, MA, USA) and allowed to dry before dot immunobinding assay. Following protein spotting, membranes were blocked for 1 h at room temperature in 5% w/v non-fat milk (Santa Cruz Biotechnology, Dallas, TX, USA) in TBS containing 0.1% v/v Tween-20 (TBS-T; Fisher Scientific, Hampton, NH, USA). Membranes were then incubated in the indicated primary antibody (in 5% milk/TBS-T) overnight at 4 °C, washed 4x in TBS-T, incubated in species-specific horseradish peroxidase-conjugated secondary antibody (in 5% milk/TBS-T) for 1h at room temperature and then washed 4x in TBS-T. Membranes were subsequently developed with ECL Western blotting substrate (Pierce, Rockford, IL, USA) using the Fujifilm LAS-3000 developer (FujiFilm, Stamford, CT, USA). Membranes were then washed 1x in TBS-T and stripped in low pH stripping buffer (25 mM glycine HCl, pH 2.0 and 1% w/v sodium dodecyl sulfate) with vigorous shaking to remove primary and secondary antibody, washed 3x in TBS-T and blocked for 1 h (in 5% milk/TBS-T) at room temperature before probing with the next primary antibody. Integrated density of immunoreactive spots was measured using MultiGauge Software (FujiFilm) and normalized to % control (vehicle). Generation, purification and characterization of rabbit pAb A11 (anti-prefibrillar oligomers, 0.5 μg ml^{-1}) and rabbit pAb OC (anti-fibrillar oligomers and fibrils; 0.25 μg ml^{-1}) have been described previously.[35] Normalization to total APP/Aβ signal was achieved by detection of human APP transgene metabolites with the mouse mAb 6E10 (1:2000; Covance, Princeton, NJ, USA). Peroxidase-conjugated goat anti-rabbit IgG (H+L; 1:20 000; Vector Labs, Burlingame, CA, USA) or goat anti-mouse IgG (H+L; 1:20 000; Vector Labs) were used for detection. To quantify monomeric Aβ levels, human Aβ 1-40/1-42 ELISA kits (Wako, Richmond, VA, USA) were used according to the manufacturer's instructions. Absolute concentrations of monomeric Aβ40 and Aβ42 or oAβ were normalized to initial tissue weight before analysis.

Neurogenesis assays
Animals were deeply anesthetized and perfused transcardially with a 0.9% saline solution followed by 4% paraformaldehyde solution. Brains were extracted and postfixed in 4% paraformaldehyde overnight at 4 °C. Brains were then transferred and equilibrated in a phosphate-buffered 30% sucrose solution 4 °C. Free-floating 40-μm coronal sections were collected using a sliding microtome and were stored in tissue cryoprotectant solution at − 20 °C until used. Immunohistochemistry was performed on a one-in-six series of 40-μm sections. Sections were immunostained as previously described.[36] For BrdU detection, sections were pretreated with 2 N HCl for 30 min at 37 °C followed by a 0.1 M borate buffer wash for 10 min. Primary antibodies used were mouse anti-neuronal-specific nuclear protein (NeuN) antibody (1:10; clone A60), rat anti-BrdU (1:250; Accurate, Westbury, NY, USA), and rabbit anti-Ki67 (1:100; Covance). Sections were also stained with 4,6-diamidino-2-phenylindole (1:5000; Sigma-Aldrich, St Louis, MO, USA). Every section was analyzed using a Zeiss LSM 710 laser scanning confocal microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA) and a 40x oil

objective lens. Cell quantification was limited to the hippocampal granule cell layer and the subgranular zone of the dentate gyrus. The proportion of BrdU cells displaying a neuronal phenotype was determined by scoring the colocalization of BrdU with NeuN, using z-stack images. The total number of BrdU and Ki67 cells was quantified by counting all cells in the hippocampal granule cell layer and subgranular zone in a one-in-six series. Resulting numbers were multiplied by six to obtain the estimated total number of BrdU and Ki67 cells per hippocampus.

Statistics

In all instances, the Shapiro–Wilk test of normality and Levene's test for homogeneity of variance were utilized for inclusion in parametric

tests ($P > 0.05$ for Shapiro–Wilk and Levene's tests). Independent sample t-tests (parametric design) or Mann–Whitney U-tests (non-parametric design) were utilized to determine significant mean differences between two groups of biological relevance. Significance for t-tests is reported with a $P \leq 0.05$ using two-tailed tests with an α-level of 0.05. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).

Additional information is available in Supplementary Materials and Methods.

Figure 1. Structures of BCI-632 and BCI-838. For BCI-632, R1 is H. For BCI-838, R1 is n-heptyl.

RESULTS

Animals were analyzed in random sequence. The mice used for the behavioral assays were the identical individual mice that were subsequently killed and used for the biochemical and histological assays. The operator(s) for biochemistry, immunohistochemistry, behavioral analyses and neurogenesis assays were blinded to the group assignments until the full data sets were available, at which point, data and mouse identities were exchanged among the various team members at the Mount Sinai and Salk sites.

BCI-838 reduced anxiety and corrected learning and memory deficits in Dutch APP transgenic mice

The synthesis and bioavailability of Group II mGluR antagonist BCI-632 (also known as MGS0039) and its prodrug BCI-838 (also
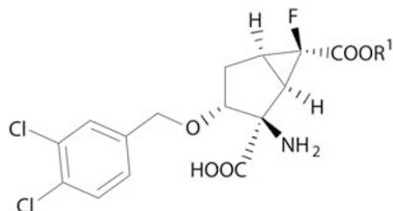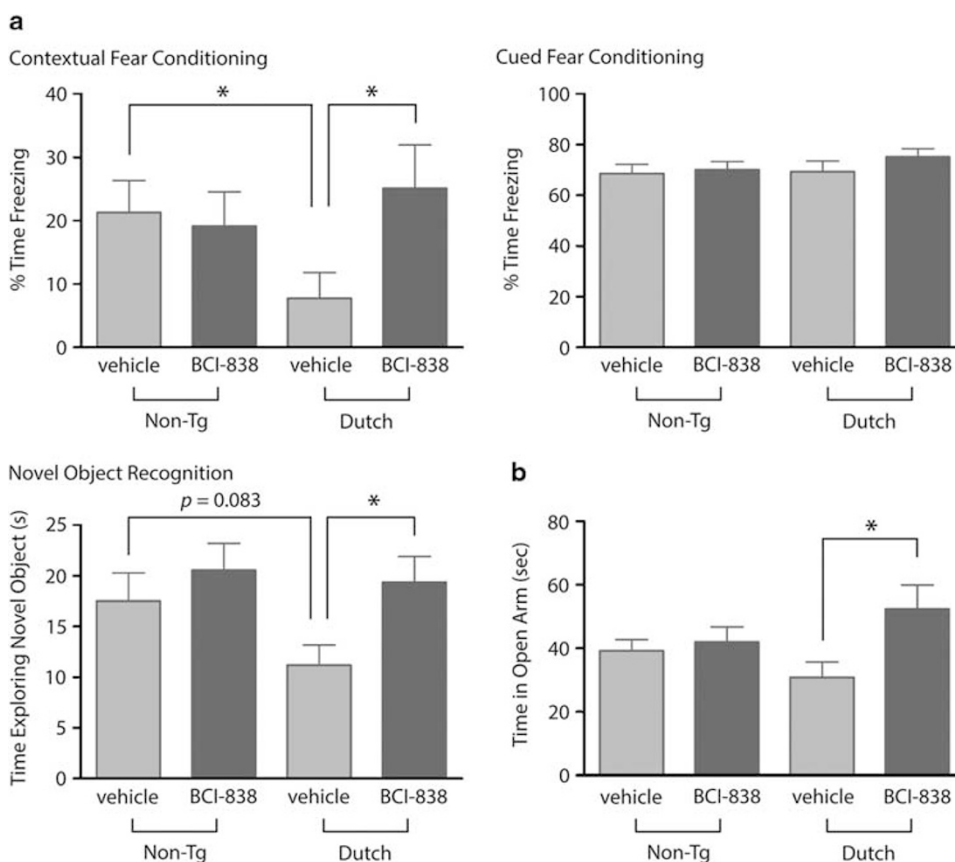

Figure 2. Chronic BCI-838-treatment for 3 months corrected cognitive deficits and reduced anxiety in amyloid precursor protein (APP) transgenic mice. (**a**) APP transgenic mice (APP E693Q) and wild-type littermates (7–10 months old) were given BCI-838 or vehicle p.o. for 3 months. BCI-838 treatment was associated with improved memory in both contextual conditioning and novel object recognition tasks (% time freezing where more freezing time means better memory; (contextual fear conditioning, $n = 14$–18; $P = 0.048$ NonTg+Veh vs Tg+Veh; $P = 0.036$ Tg+Veh vs Tg+BCI-838); (cued fear conditioning, $n = 14$–18): time exploring novel object(s) where more time spent at novel object(s) means better memory; (novel object recognition, $n = 14$–18; $P = 0.083$ NonTg+Veh vs Tg+Veh; $P = 0.017$ Tg+Veh vs Tg+BCI-838)). (**b**) Reduction in anxiety was seen in BCI-838-treated APP transgenic mice in the elevated plus maze (% time in open arm where more time in open arm means less anxiety; $n = 12$–18; $P = 0.024$ Tg+Veh vs Tg+BCI-838). Data are presented as means ± s.e.m. *$P < 0.05$.
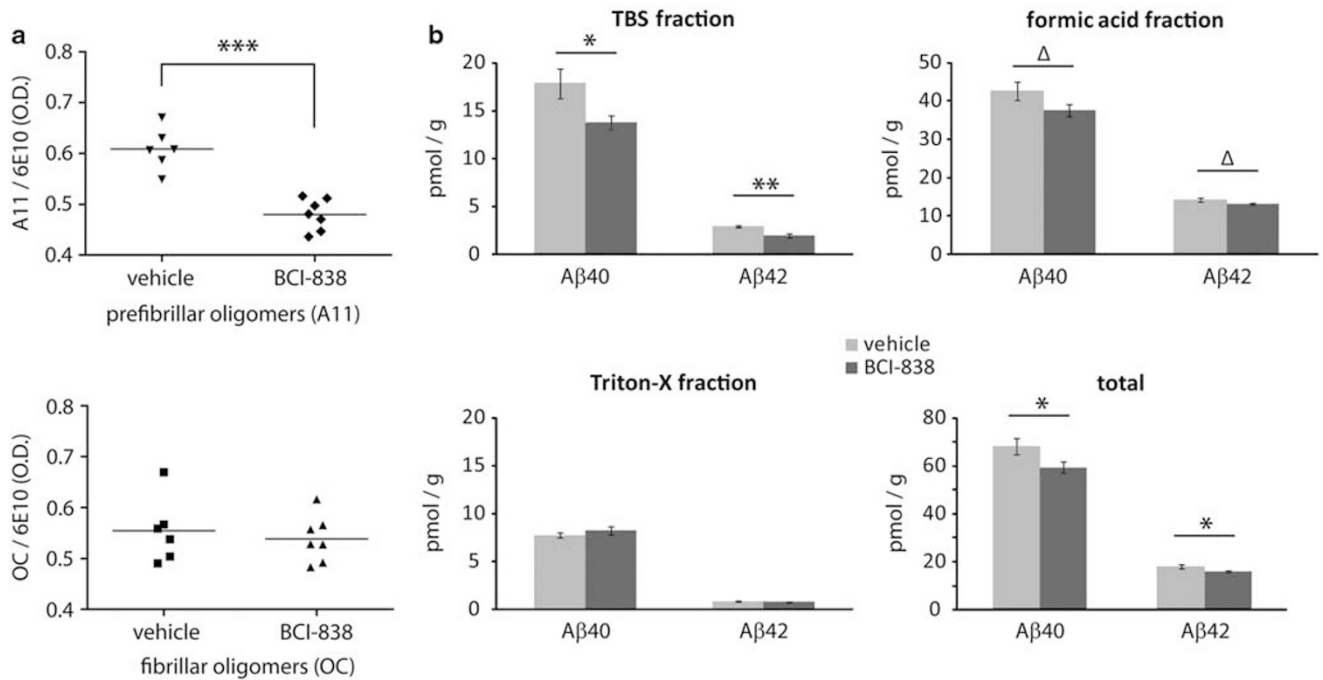
Figure 3. Chronic suppression of Group II mGluRs for 3 months lowered cortical Aβ oligomer and hippocampal Aβ42 levels *in vivo* in amyloid precursor protein (APP) transgenic mice (APP E693Q). (**a**) APP E693Q mice (7–10 months old) were treated with BCI-838 for 3 months; then Aβ oligomer levels were measured by dot blots using two antibodies (A11, OC) that recognize different conformations of Aβ oligomers. A11-reactive Aβ oligomer level was reduced in cortex, while there was no change in the level of OC-reactive ones. (**b**) Decreases in soluble (tris-buffered saline (TBS) fraction) and total Aβ42 levels were observed in hippocampus after the 3 months of treatment. $n = 6$–7 for each. Data are presented as means ± s.e.m. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Δ $P < 0.1$ (trend).

known as MGS0210) (Figure 1) have been reported.[37,38] Group II mGluR antagonists have drawn attention as potential therapeutic compounds for the treatment of depression, anxiety and cognitive disorders. Inhibition of Group II mGluR in rodents enhances working memory, spatial learning and social recognition,[10,39] while also exhibiting acute and chronic antidepressant-like/anxiolytic effects.[9,11,40,41] As AD patients suffer from cognitive impairment, depression and anxiety,[42,43] Group II mGluR antagonists are potentially attractive interventional agents for AD as well.

A single dose of Group II mGluR antagonist is apparently sufficient for many acute effects, and most of the studies cited above were performed following a single administration of the antagonist. As our initial strategy was focused on Aβ reduction, we used primarily chronic treatment protocols. However, we were especially concerned that chronic suppression of a specific receptor activity would induce compensatory mechanisms (for example, homeostatic synaptic plasticity) that may weaken or abolish the acute effects of the drug when used over a period of months. With these issues in mind, we chose chronic treatment for 3 months to investigate whether BCI-838 benefits were still achievable long-term in wild-type and Aβ-oligomer-forming Dutch APP transgenic mice (APP E693Q).[33] These mice were chosen because they accumulate only oAβ and no fibrillar Aβ. The E693Q mutation preferentially stabilizes an oAβ conformational state, perhaps by destabilizing fibrillogenic Aβ conformations.[33] We would like to point out that the relevance of the conformation of E693Q oAβ to that of wild-type oAβ remains to be determined.[33] Chronic BCI-838 treatment for 3 months in Dutch APP transgenic mice rescued transgene-induced learning and memory deficit in both contextual fear conditioning and novel object recognition tasks (Figure 2a) and reduced anxiety in the elevated plus maze test (Figure 2b). In contrast, no negative or positive behavioral effects were observed following chronic BCI-838 administration to wild-type mice.

Chronic BCI-838 administration lowered Aβ monomer and oligomer levels *in vivo*

In an isolated intact nerve terminal system, Group II mGluR stimulation was directly linked to Aβ42 production; that is, the antagonists suppress synaptic Aβ42 production and release.[7] One goal of the current *in vivo* study was to assess whether global inhibition of Group II mGluRs would lead to reduction of monomeric and/or oAβ levels in cortex and/or hippocampus, brain regions prominently devastated by the neuropathology of AD. Brain extracts were serially fractionated to isolate TBS-soluble, Triton-X-soluble and formic acid-soluble fractions. Aβ oligomer levels were determined in TBS-soluble fractions by dot blots using two conformation-specific antibodies A11 and OC, which recognize prefibrillar and fibrillar oligomers, respectively.[35] We tested an extended drug administration period of 3 months in Dutch APP transgenic mice (APP E693Q).[33] The drug treatment was associated with a reduction in prefibrillar oligomer levels in the cortex (Figure 3a) and in soluble and total Aβ42 levels in the hippocampus (Figure 3b; Supplementary Table 1) without affecting the expression of the mutant APP transgene (Supplementary Figure 1). Despite having attained statistical significance, these differences were modest, and their importance is uncertain. Similar results were obtained in a second line of mice that contained not only the Dutch E693Q APP mutation but also the exon 9 deleted mutant form of PS1 (Supplementary Figure 2). These APP E693Q x PS1 Δ exon 9 form both oligomers and fibrils of Aβ.

Hippocampal neurogenesis was disproportionately enhanced in Dutch APP transgenic mice after chronic administration of BCI-838

The hippocampus is one of the first regions of the brain affected by amyloid pathology in AD,[44] and several studies have indicated that hippocampal neurogenesis is impaired as part of the pathogenesis of AD.[30] Adult hippocampal neurogenesis is thought
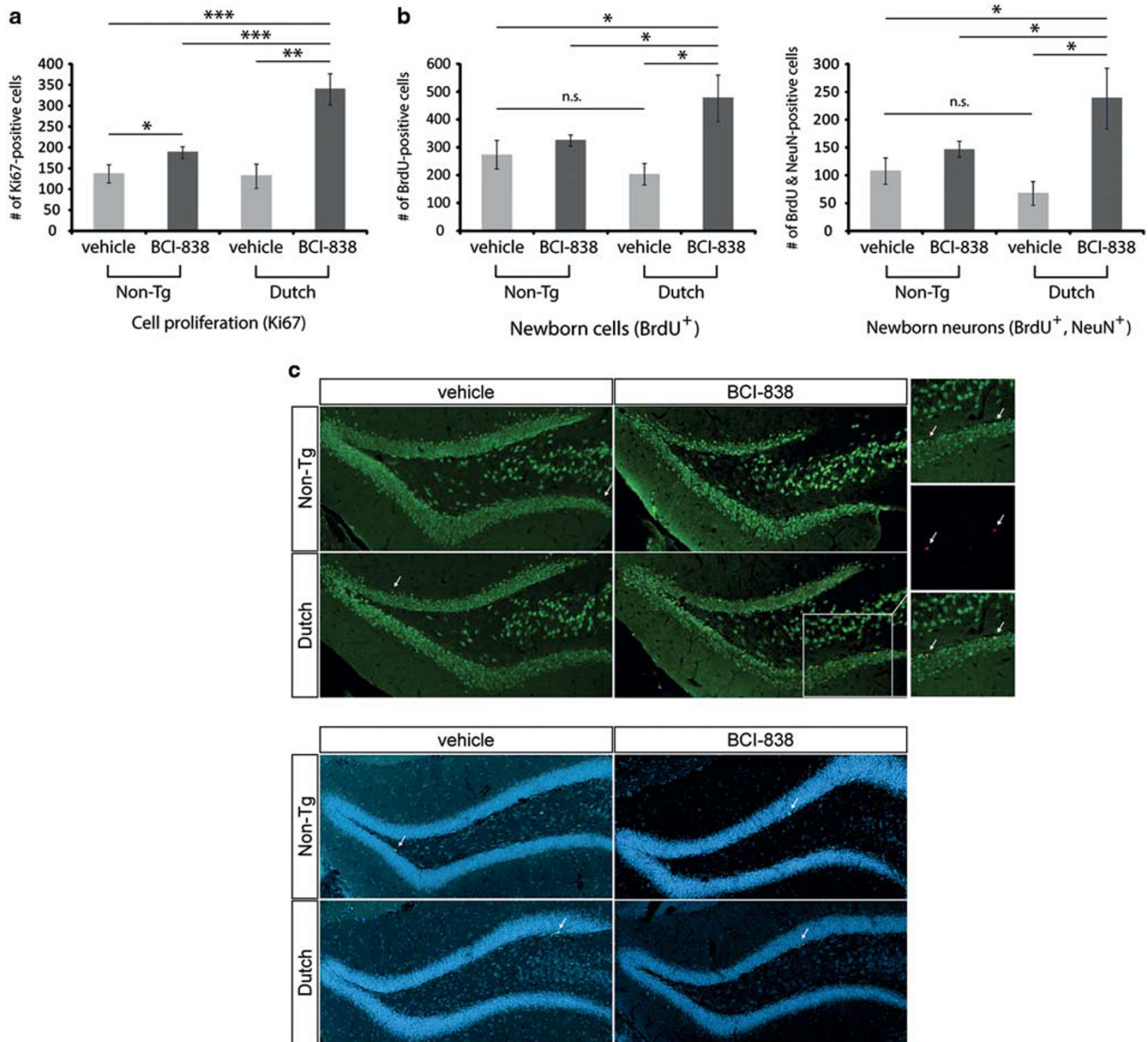
Figure 4. BCI-632 stimulated hippocampal neurogenesis in amyloid precursor protein (APP) transgenic mice. (**a**) APP transgenic mice (APP E693Q) and wild-type littermates (7–10 months old) were given BCI-838 or vehicle p.o. for 3 months. BrdU (50 mg kg^{-1}) was intraperitoneally injected on days 17–24 to label newborn cells. The total number of Ki67-positive cells per hippocampus was increased in both wild-type and APP transgenic mice that were treated with BCI-838. Ki67 is expressed in all proliferating cells in the active phases of the cell cycle. (**b**) There was a significantly increased total number of newborn cells (BrdU-positive) and newborn neurons (BrdU- and NeuN-positive) per hippocampus in the drug-treated APP transgenic mice. NeuN was used as a mature neuron marker. $n = 10$, 12, 5, 7, respectively. Data are presented as means ± s.e.m. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS, not significant. (**c**) In the upper set of pictures, coronal sections of the dentate granule cell layer are stained with NeuN (mature neurons—green) and BrdU (newborn cells—red). Arrows point to BrdU cells in the granule cell layer. Inset highlights two BrdU cells (BrdU only—left cell, BrdU colabeled with NeuN—right cell) in the APP transgenic mice treated with BCI-838. In the lower set of pictures, coronal sections of the dentate granule cell layer are stained with Ki67 (cell proliferation—green) and 4,6-diamidino-2-phenylindole (DAPI; blue). Arrows identify a few of the Ki67 cells present in the dentate gyrus.

to have a role in learning and memory,[45] and manipulations to enhance adult neurogenesis, if administered early in the disease process, could conceivably delay the appearance of the cognitive deficits associated with AD or reduce the severity of the deficits.

Adult hippocampal neurogenesis consists of cell proliferation, neuronal differentiation and cell survival. To study how BCI-632 affects hippocampal neurogenesis and survival of newborn cells *in vivo* in Dutch APP transgenic mice, we injected BrdU (50 mg kg^{-1}) on days 17–24 of the 3-month treatment with BCI-838. BrdU, a thymidine analog, is incorporated into actively dividing cells, and quantifying the number of BrdU cells at a later time point (2 months) can be used as an indicator of cell survival. BCI-632 stimulated cell proliferation in both wild-type and Dutch APP transgenic mice, as indicated by Ki67-positive cells (Figures 4a and c). In addition, treatment with BCI-838 resulted in significantly increased numbers of surviving, newly born neurons (BrdU$^+$/

NeuN[+]) in the hippocampi of Dutch APP transgenic mice but not in the hippocampi of wild-type mice (Figures 4b and c). Unexpectedly, the fold-increase in hippocampal neurogenesis in response to BCI-838 tended to be greater in the Dutch APP mice than the corresponding effect in the wild-type mice (Figure 4). A similar phenomenon was observed using a structurally unrelated proneurogenic compound to treat mice overexpressing APP.[14]

DISCUSSION

Herein, we assessed the therapeutic potential of chronic pharmacological inhibition of Group II mGluRs in an APP transgenic mouse model that develops impaired learning behavior in relation to accumulation of mutant Aβ oligomers that never form amyloid plaques.[46] Once-daily dosing of the orally bioavailable prodrug, BCI-838, delivers a sufficient brain concentration of its active metabolite BCI-632 to inhibit Group II mGluR for 22 h. Three months of treatment with BCI-838 provided anxiolytic effects, reversed Dutch APP transgene-associated learning and memory impairment, and decreased the levels of monomeric and oAβ peptides in the hippocampus and cortex of the two different AD mouse models. Notably, BCI-838 administration stimulated hippocampal progenitor cell proliferation in both wild-type and AD mice for 3 months, which resulted in significantly increased numbers of newborn neurons in the hippocampi of Dutch APP transgenic mice. In addition to treatment, the proneurogenic properties make the compound attractive for potential use in reversing some of the early symptoms of AD, possibly through reparative effects of the newborn neurons.

To assess the possible contribution of a prosurvival effect of BCI-838 on newborn neurons, we injected BrdU early in the course of drug treatment (days 17–24) and allowed over 2 months of maturation/survival to pass between injection and analysis. The significant increase in the numbers of total newborn neurons in APP transgenic mouse hippocampi may be attributed to a combination of stimulated cell proliferation and enhanced cell survival. In contrast, 3 months of BCI-838 treatment led to an apparent stimulation of cell proliferation but not newborn neuron survival in wild-type mice, suggesting that most newborn cells do not mature and integrate into the existing circuitry. This finding correlates with the absence of a behavioral response to drug in wild-type mice treated with BCI-838. Overall, the robustness of the neurogenesis effect led us to conclude that the behavioral benefits in the Dutch APP transgenic mice are probably due, at least in part, to enhanced hippocampal neurogenesis.

Modulation of learning and anxiety by Group II mGluR antagonists is well documented,[9–12] and these phenomena are believed to be related to the stimulation of neurogenesis by these drugs. However, the molecular mechanisms linking Group II mGluRs to neurogenesis remain to be fully elucidated. Further research will be required to determine which of these events is/ are relevant to this particular picture of Group II mGluR antagonist-induced changes in learning, anxiety, Aβ metabolism and neurogenesis. Our next step in elucidating the key actions of BCI-838 will involve the use of cranial irradiation to impair hippocampal neurogenesis. If hippocampal neurogenesis is key to the beneficial effects of BCI-838 in Dutch APP transgenic mice, then cranial irradiation before drug treatment should abolish the drug's benefit.[47]

Patients who are not demented but have smaller hippocampal volumes and higher rates of shrinkage are two to four times as likely to develop dementia, suggesting that considerable hippocampal cell loss has already occurred at the point of mild cognitive impairment, a milder memory disorder that represents a prodromal form of AD.[48] Thus, stimulating hippocampal neurogenesis in some mild cognitive impairment patients may have the potential to slow or even reverse some cognitive defects or

anxiety. A recent study revealed that the maturation time of new dentate granule cells exceeds 6 months in monkeys and is expected to be longer in humans,[49] which implies that it could take months to see the full benefits of proneurogenic drug treatments. The drug could potentially possess cholinergic properties, as there are links between the metabotropic and cholinergic systems.[50,51] Although this could explain in part improved learning behavior, a cholinergic agonist would not be predicted to be responsible for the proneurogenic effect of mGlu2/3 antagonists.

A standard registration-enabling toxicological package for BCI-838 has been performed in rats, marmosets and cynomologous monkeys for 14 days (at 10–300 mg kg^{-1} day^{-1}) without the emergence of serious adverse events. Body weight, food intake and water intake were unaffected by drug administration, and no clinical or anatomic pathology attributable to BCI-838 was revealed following standard examinations.[52,53] The pharmacokinetics, safety and tolerability of BCI-838 have been evaluated in a phase 1 program that included a single ascending dose study in healthy male subjects and a multiple ascending dose study in healthy male and female subjects.[52,53] The results of these studies indicate that the prodrug BCI-838 effectively delivers its active metabolite, BCI-632. At both single and multiple doses, the safety data generated in these studies indicate that the drug is well tolerated in healthy subjects, with an increase in drug-related mild to moderate adverse events reported at the highest dose levels. There was no evidence of clinically relevant alterations in vital signs, electrocardiograms or laboratory parameters attributable to BCI-838 and no serious adverse events were observed (unpublished observations and Gadient et al.[32]).

In conclusion, our findings raise the possibility that chronic pharmacological inhibition of Group II mGluR has the potential to be a disease-modifying treatment for AD that targets cognitive/emotional defects and modulates neurogenesis. There are several potential trial designs that can be contemplated to test the effects of BCI-838 in AD. For example, it would be of great interest to see whether BCI-838 can also be used to treat some mild cognitive impairment patients in order to slow or reverse progression to dementia because of its proneurogenic activity. Finally, it is also conceivable that BCI-838 may be useful not only in the treatment phase of the illness but also in a prevention protocol.[54] Taking BCI-838 before pathology begins may represent the best chance of staving off the incapacitation and eventual decortication that define AD.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)