

The role of G protein-coupled receptors in the pathology of Alzheimer's disease

Amantha Thathiah and Bart De Strooper

Abstract | G protein-coupled receptors (GPCRs) are involved in numerous key neurotransmitter systems in the brain that are disrupted in Alzheimer's disease (AD). GPCRs also directly influence the amyloid cascade through modulation of the α -, β - and γ -secretases, proteolysis of the amyloid precursor protein (APP), and regulation of amyloid- β degradation. Additionally, amyloid- β has been shown to perturb GPCR function. Emerging insights into the mechanistic link between GPCRs and AD highlight the potential of this class of receptors as a therapeutic target for AD.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of proteinaceous aggregates and neurofibrillary lesions composed of the amyloid- β peptide and the hyperphosphorylated microtubule-associated protein tau, respectively. Current AD therapies mainly target acetyl cholinesterase (AChE), which broadly stimulates cholinergic neurons. However, neurodegeneration is not limited to a specific neurotransmitter system. Glutamatergic, serotonergic, adrenergic and peptidergic neurotransmitter systems are also deregulated in AD. Impaired neuronal signalling may affect the proteolysis of the amyloid precursor protein (APP) and promote amyloid formation in the AD brain. In addition, amyloid toxicity and neurodegeneration may affect neurotransmission, suggesting the involvement of complex positive-feedback loops in the pathogenesis of the disease. Consequently, a complementary approach that includes stimulation or promotion of neurotransmission in addition to lowering the amyloid burden is an attractive therapeutic strategy for the treatment of AD.

Several studies have presented compelling evidence implicating G protein-coupled receptors (GPCRs) in the pathogenesis of AD and in multiple stages of the processing of APP. Sequential cleavage of APP by the α -, β - and γ -secretases, which are regulated by GPCRs, determines the extent of amyloid- β peptide generation (FIG. 1), and amyloid- β can directly or indirectly affect GPCR function. In this Review, we discuss the GPCRs that have been implicated in cholinergic, glutamatergic, adrenergic and serotonergic dysfunction in AD, focusing on GPCR modulation of the α -, β -, and γ -secretases, amyloid- β deposition and amyloid plaque formation.

We address the involvement of GPCRs in the amyloid cascade and pharmacological approaches to target the putative therapeutic properties of AD-associated GPCRs. We also briefly discuss the role of GPCRs in amyloid- β -mediated toxicity and neuroinflammation.

Regulation of α -secretase

Three enzymes, belonging to the ADAM (a disintegrin and metalloproteinase) family — ADAM9, ADAM10 and ADAM17 — are putative α -secretases (reviewed in REF. 1). Cleavage of APP by α -secretase occurs within the amyloid- β peptide sequence, precluding amyloid- β generation and producing the soluble amino-terminal ectodomain of APP (sAPP α) and a membrane-anchored 83-amino acid carboxy-terminal fragment (C83) (FIG. 1). Subsequent cleavage of C83 by the γ -secretase complex yields the APP intracellular domain (AICD) and a short fragment termed p3. sAPP α has neurotrophic and neuroprotective properties^{2,3} and enhances long-term potentiation (LTP)⁴. Lower levels of sAPP α have also been found in the cerebrospinal fluid (CSF) of patients with AD⁵, suggesting that decreased α -secretase activity may contribute to the development of AD. Thus, α -secretase has therapeutic potential, although further work is needed to evaluate the consequences of increased α -secretase activity in the brain. Of note, it remains unclear whether p3 is truly innocuous⁶ as is generally assumed.

The α -secretase-mediated cleavage of APP is regulated by protein kinase C (PKC)^{7,8}, cyclic AMP-protein kinase A (PKA)^{9–11}, mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK)¹² and phosphatidylinositol 3-kinase (PI3K)¹³. Specifically,

Department for Molecular and Developmental Genetics, Flanders Institute for Biotechnology (VIB), Leuven, Belgium, and Center for Human Genetics, Catholic University of Leuven, Leuven, Belgium.
e-mails: Bart.Destrooper@cme.vib-kuleuven.be; Amantha.Thathiah@cme.vib-kuleuven.be
doi:10.1038/nrn2977

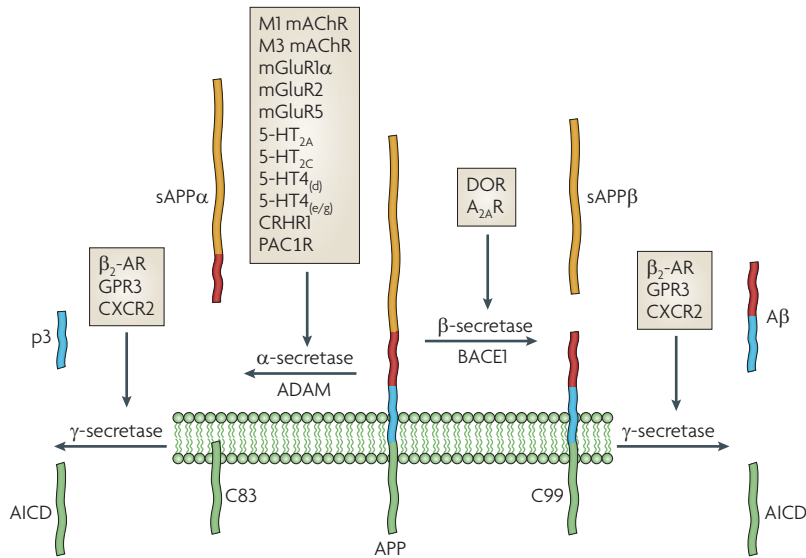


Figure 1 | Modulation of APP processing by GPCRs. Cleavage of amyloid precursor protein (APP) by α -secretase generates the soluble amino-terminal ectodomain of APP (sAPP α) and the carboxy-terminal fragment C83. Subsequent cleavage of C83 by the γ -secretase complex yields the APP intracellular domain (AICD) and a short fragment termed p3. Several G protein-coupled receptors (GPCRs), including muscarinic, metabotropic and serotonergic receptors modulate α -secretase-mediated proteolysis. Alternatively, cleavage of APP by β -secretase generates sAPP β and the C-terminal fragment C99. Subsequent cleavage of C99 by the γ -secretase complex yields the AICD and the amyloid- β peptide. Of the GPCRs that regulate this processing, the δ -opioid receptor (DOR) and the adenosine A_{2A} receptor ($A_{2A}R$) have been shown to modulate β -secretase-mediated cleavage of APP, whereas the β_2 adrenergic receptor (β_2 -AR), G protein-coupled receptor 3 (GPR3), and CXCR-chemokine receptor 2 (CXCR2) have been shown to modulate γ -secretase-mediated cleavage of C99 or C83. A β , amyloid- β ; ADAM, a disintegrin and metalloproteinase; BACE1, β -site APP-converting enzyme 1; CRHR1, corticotrophin-releasing hormone (CRH) receptor type I; 5-HT, 5-hydroxytryptamine (serotonin); mAChR, muscarinic acetylcholine receptor; mGluR, metabotropic glutamate receptor; PAC $_1$ R, pituitary adenylate cyclase 1 receptor.

activation of these signalling cascades shifts APP metabolism towards the α -secretase-mediated pathway and away from β -secretase-mediated amyloid- β generation^{14,15} (FIG. 1). Conversely, a recent study suggests that chronic, rather than acute, activation of PKC differentially regulates the PKC α and PKC ϵ isozymes, leading to increased amyloid- β generation¹⁶. Nevertheless, numerous studies have suggested that GPCRs and activation of their downstream signal cascades increases the non-amyloidogenic processing of APP.

Muscarinic acetylcholine receptors. Muscarinic acetylcholine receptors (mAChRs), a family of five receptor subtypes (M1–M5)^{17,18}, have been implicated in the pathophysiology of major diseases of the CNS, including AD¹⁹ (BOX 1). Agonist-induced activation of the M1 and M3 mAChRs, which are coupled to phosphoinositide hydrolysis and PKC activation, stimulates sAPP release *in vitro*^{20,21}, implicating mAChR activation in increased α -secretase activity (FIG. 2). This effect is blocked by treatment with a PKC inhibitor or a muscarinic antagonist^{20,21}. Furthermore, the effect can be mimicked by phorbol esters, which directly activate

PKC and stimulate increased release of sAPP¹⁵ and p3 (REF. 14), and decreased amyloid- β generation^{14,15}.

The M1 mAChR is the most abundant subtype in the cortex and hippocampus^{17,18,22}, two major brain regions that develop amyloid plaques and neurofibrillary tangles (NFTs) in AD. Postsynaptic M1 mAChRs also play a major part in hippocampus-dependent learning and memory and, in particular, short-term memory and memory consolidation²³, which is impaired in AD^{24–26}. Therefore, considerable efforts have been directed towards developing M1 mAChR-selective agonists that are capable of restoring the cognitive deficits in patients with AD. In a mouse model of AD, a selective M1 mAChR agonist, AF267B, reduces the amyloid- and tau-related pathologies in the hippocampus and cerebral cortex, and rescues impairments in hippocampus-dependent learning and memory²⁷. The effect on amyloid- β seems to be mediated by an increase in PKC activation, ERK1 and ERK2 phosphorylation, and an increase in ADAM17 expression, whereas the effect on tau is mediated by a reduction in glycogen synthase kinase 3 β (GSK3 β) activity and a corresponding reduction in tau phosphorylation²⁷. Thus, the development of M1-selective agonists for the treatment of AD could provide a symptomatic and a disease-modifying treatment in one compound.

Genetic ablation studies provide additional support for the development of an M1-specific therapy, as inactivation of the M1 mAChR leads to a strong increase in amyloid- β generation and amyloid plaque formation in a mouse model of AD²⁸. Although it has been difficult to synthesize fully specific M1 mAChR agonists, an allosteric M1 mAChR agonist, TBPB, was recently reported to be highly selective for the M1 mAChR in rodents²⁹. Perhaps most importantly, M1-selective therapy would avoid the general increase in ACh levels that follows administration of AChE inhibitors, which activates all mAChRs, including the M2 and M4 mAChRs — the receptor subtypes that inhibit sAPP α release and aggravate amyloid- β generation^{28,30} — negating the beneficial effects of M1 mAChR stimulation.

Metabotropic glutamate receptors. Treatment of primary neuronal cultures and brain slices with a general metabotropic glutamate receptor (mGluR) agonist stimulates sAPP secretion³¹, suggesting that one or more mGluRs are linked to the processing of APP by α -secretase. This has prompted further investigation into the involvement of specific mGluR subtypes in the pathogenesis of AD.

Based on their pharmacology, sequence homology, G protein coupling and association with specific second-messenger systems, mGluRs are divided into three groups: group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8)³². Group I mGluRs are positively coupled to phospholipase C (PLC)³³ and participate in the regulation of synaptic plasticity³⁴ and postsynaptic glutamatergic excitability³⁵. Group II and group III mGluRs are negatively coupled to adenylyl cyclase, inhibit cAMP production and activate the MAPK and PI3K pathways^{36,37}. Group I mGluR-linked PLC activity is downregulated in the cerebral cortex

Box 1 | The cholinergic and amyloid cascade hypotheses

The amyloid cascade hypothesis

The amyloid cascade hypothesis postulates that gradual changes in the metabolism and aggregation of amyloid- β initiates a cascade of neuronal and inflammatory injury that culminates in extensive neuronal dysfunction and cell death associated with neurotransmitter deficits and dementia^{145,146}.

The cholinergic hypothesis

The cholinergic hypothesis posits that a dysfunction in acetylcholine (ACh)-containing neurons substantially contributes to the cognitive decline observed in Alzheimer's disease (AD)¹⁴⁷. This is based on the observation that cholinergic transmission has a fundamental role in cognition and is disrupted in patients with AD^{148,149}.

Convergence of the amyloid cascade and cholinergic hypotheses

ACh is a key neurotransmitter involved in learning and memory¹⁵⁰ that binds to distinct receptor subtypes in the brain: nicotinic ACh receptors (nAChRs) and muscarinic ACh receptors (mAChRs). Nicotinic neurotransmission is implicated in the pathogenesis of AD (TABLE 1). Additional evidence suggests that the major mAChR subtypes involved in AD are the postsynaptic M1 mAChRs, which mediate the effects of ACh, and the presynaptic M2 mAChRs, which inhibit ACh release^{151,152}.

Amyloid- β deposition may contribute to the cholinergic dysfunction in AD by decreasing the release of presynaptic ACh and impairing the coupling of postsynaptic M1 mAChRs with G proteins. This leads to decreased signal transduction, impairments in cognition, a reduction in the levels of amyloid precursor protein (APP), the generation of more neurotoxic amyloid- β and a further decrease in ACh release¹¹¹. Genetic ablation of the M1 mAChR in a transgenic mouse model of AD decreases the production of the soluble amino-terminal ectodomain of APP (sAPP α), increases amyloid- β generation and exacerbates the amyloid plaque pathology²⁸, supporting the development of M1-selective agonists. In addition, M1 mAChR activation reduces tau phosphorylation^{27,153} and alleviates hippocampus-dependent memory impairments²⁷, making M1 mAChRs a compelling therapeutic target for AD. Furthermore, receptor subtype specificity will be of key importance as M2 and M4 mAChRs seem to inhibit sAPP α release and potentially aggravate amyloid- β generation^{28,30}, and activation of nAChRs exacerbates the tau pathology¹⁵⁴.

of patients with AD³⁸, and *in vitro* data indicate that mGluR5 stimulation increases the translation of APP mRNA³⁹. By contrast, mGluR2 is overexpressed in the hippocampus of patients with AD⁴⁰. Interestingly, mGluR2 stimulation leads to ERK activation, tau phosphorylation and a reduction in oxidative stress-induced neuronal cytotoxicity⁴¹ (FIG. 2).

A recent study demonstrates that group I mGluR stimulation of synaptoneuroosomes leads to activation of α - and β -secretases (based on the accumulation of C83 and C99, respectively) and increased release of amyloid- β_{40} (REF. 42), the 40-amino acid isoform of amyloid- β that is anti-amyloidogenic *in vivo*⁴³. Similar to group I mGluRs, group II mGluR stimulation activates the α - and β -secretases. However, group II mGluR stimulation elicits the release of amyloid- β_{42} — the 42-amino acid isoform of amyloid- β that aggregates into amyloid much more rapidly than amyloid- β_{40} *in vitro* (reviewed in REF. 44) — which is the predominant isoform of amyloid- β that accumulates in the brains of patients with AD and is essential for seeding amyloid- β deposition *in vivo*^{45,46}. The group II mGluR-mediated increase in amyloid- β_{42} could be blocked with a specific group II mGluR antagonist, with only a transient induction of amyloid- β_{40} generation⁴². Thus, upregulation of group I mGluR signalling may increase synaptic amyloid- β_{40} generation, whereas downregulation of group II mGluR signalling may decrease synaptic amyloid- β_{42} generation, supporting the inhibition of group II mGluRs as a therapeutic approach for AD. Indeed, group II mGluR inhibitors enhance hippocampus-dependent cognitive functions in rodents⁴⁷, although the effect on amyloid- β generation remains to be determined. It is also unclear how group II mGluRs and group I mGluRs produce differential effects on amyloid- β_{40} and amyloid- β_{42} release.

5-hydroxytryptamine receptors. Several lines of evidence suggest that 5-hydroxytryptamine (5-HT; also known as serotonin) signalling is impaired in AD, and studies in animal models have shown the potential of this receptor system — in particular the 5-HT₂, 5-HT₄ and 5-HT₆ receptor subtypes — as therapeutic targets to address the cognitive deficits in AD.

5-HT stimulates sAPP release through activation of the 5-HT_{2A} and 5-HT_{2C} receptors. 5-HT_{2A} receptor binding is decreased in the AD brain⁴⁸, and polymorphic variations have been described for the 5-HT_{2A} gene that may be risk factors for hallucinations⁴⁹, aggression⁵⁰ and major depression⁵¹ in AD. Although there is evidence to suggest that the 5-HT_{2A} and 5-HT_{2C} receptors modulate sAPP secretion *in vitro*⁵² and *in vivo*⁵³ (FIG. 2), further studies are required to determine whether this effect is mediated by a change in α - or β -secretase activity and whether this effect correlates with a change in amyloid- β generation.

The 5-HT₄ receptors are highly expressed in the hippocampus, basal ganglia and amygdala⁵⁴, and might also be involved in the memory and cognition defects in AD. Application of 5-HT to Chinese hamster ovary (CHO) cells that stably express the 5-HT₄ receptor enhances sAPP α release¹⁰. Similarly, *in vivo* administration of prucalopride, a 5-HT₄ agonist, to C57BL/6j mice and an AD transgenic mouse model leads to an increase in sAPP α levels in the hippocampus and cortex. This effect is blocked by pretreatment with the 5-HT₄ receptor antagonist GR125487 (REF. 55). Pharmacological activation of the 5-HT₄ receptor also stimulates ACh release in the rat frontal cortex and improves cholinergic function⁵⁶, which is important for memory acquisition and retention. Interestingly, activation of the 5-HT₄ receptor leads to a reduction in amyloid- β generation in neuronal cultures from a mouse model of AD⁵⁷.

Synaptoneurosome

A purified synapse, containing a presynaptic sac (synaptosome) attached to a resealed postsynaptic sac (neurosome), that is modestly enriched for synaptic proteins.

Table 1 | **G protein-coupled receptors reported to be involved in Alzheimer's disease**

Receptor	Subtype	Agonist or antagonist	Second messenger	Mode of action	Relevance to AD	Refs
mAChR	M1 or M3 mAChR	Carbachol	↑PLC, PKC and DAG ↑PIP2 hydrolysis	α-secretase (unconfirmed)	↑sAPP and ↓Aβ	15, 20, 21
		AF267B	↑PKC, ERK1 and ERK2 ↓GSK3β	↑ADAM17	↓Aβ ₄₂ and tau No effect on Aβ ₄₀	27
		AF102B	↑PIP2 hydrolysis	α-secretase (unconfirmed)	↓Aβ	177, 178
		TBPB	ND	α-secretase (unconfirmed)	↑sAPPα and ↓Aβ ₄₀	29
Group I mGluR	mGluR1 or mGluR5	DHPG	ND	α- or β-secretase	↑C83, C99 and Aβ ₄₀	42
	mGluR1α	ACPD	↑PLC ↑PIP2 hydrolysis	α-secretase (unconfirmed)	↑sAPP and sAPPα	31, 179, 180
		Melittin	↑PLA2	α-secretase (unconfirmed)	↑sAPP	180
Group II mGluR	mGluR2 or mGluR3	DCG-IV	↑AC, MAPK and PI3K ↓cAMP	α-secretase (mainly) β-secretase (transiently)	↑C83, C99 (transiently) and Aβ ₄₂	42
	mGluR2	LY379268	↑ERK	ND	↑tau	41
5-HT ₂ R	5-HT _{2A} R	5-HT	↑PLA2 ↑PIP2 hydrolysis	α-secretase (unconfirmed)	↑sAPP and APLP2	52
		5-HT _{2C} R	5-HT	↑PLA2 and PKC ↑PIP2 hydrolysis	α-secretase (unconfirmed)	↑sAPP and APLP2
	Dexnorfenfluramine (DEXNOR)	ND	α-secretase (unconfirmed)	↓Aβ ₄₂	52, 53	
5-HT ₄ R	Prucalopride or renzapride	RS 67333	↑AC, Rac, Rap and EPAC ↑cAMP	α-secretase (unconfirmed)	↑sAPPα (in vitro and in vivo)	10, 55, 181
		ND	ND	ND	↓Aβ	57
	5-HT _{4(d)} R	Prucalopride	ND	α-secretase (unconfirmed)	↑sAPPα and ↓Aβ	182
	5-HT _{4(e/g)} R	5-HT	↓PKA(H89) ↑Rac, Rap and EPAC	α-secretase (unconfirmed)	↑sAPPα No effect on Aβ	10, 181
5-HT ₆ R	SB-74257 and SAM-531 (antagonists)*	ND	↓GABA ↑ACh and Glu	Improved cognition and memory	63, 64 (reviews)	
CRHR1	CRH	↑AC ↓NF-κB	α-secretase (unconfirmed)	↑sAPPα	67	
PAC ₁ R	PACAP	↑ERK1, ERK2, PI3K and PKC (partially, independent of MAPK)	α-secretase (ADAM10)	↑sAPPα	74	
DOR	DADLE	ND	β- and γ-secretase	↓Aβ ₄₀ and Aβ ₄₂	78	
β ₂ AR	Isoproterenol or clenbuterol	ND	γ-secretase	↑Aβ ₄₀ and Aβ ₄₂	80	
	ICI 118,551 (antagonist)	ND		↓Amyloid plaques		
GPR3	Overexpression	ND	γ-secretase	↑Aβ ₄₀ and Aβ ₄₂	92	
	Genetic ablation			↓Aβ ₄₀ and Aβ ₄₂		
CXCR2	SB-225002 (antagonist)	ND	↓γ-secretase expression	↓Aβ ₄₀ and Aβ ₄₂	99	
		↑PI3K, ERK1 and ERK2		↑tau	183	
AT ₂ R	Aβ ₄₂ treatment	ND	↑AT ₂ R oligomers ↓M1 mAChR signalling	↑tau and neurodegeneration	110, 184	

Table 1 (cont.) | G protein-coupled receptors reported to be involved in Alzheimer's disease

Receptor	Subtype	Agonist or antagonist	Second messenger	Mode of action	Relevance to AD	Refs
A _{2A} R		Caffeine (antagonist)	ND	↓β-secretase ↓PS1 expression	↓Aβ ₄₀ and Aβ ₄₂	119
		SCH 58261 (antagonist)	↑p38 MAPK		↓Aβ toxicity	118, 120, 185
CCR2		Aβ ₄₂ treatment	ND	ND	↑CCL2	127
		Genetic ablation (Tg2576)			↑Aβ ₄₀ and Aβ ₄₂	128
CX ₃ CR1		Genetic ablation (3xTg-AD)	ND	ND	↓Neuronal loss	131
GLP1R		(Val ⁶⁸)GLP1	ND	ND	↓Aβ ₄₂ or Aβ ₂₅₋₃₅ toxicity	164, 165
		Exendin-4	ND		↓Aβ ₄₀	164
		Exendin (1–9)	↑MAPK, ERK1 and ERK2		Improved cognition	172
AMY receptor		AC187 (antagonist)	↓Caspase activation (↓JNK and p38 MAPK?)	ND	↓Aβ toxicity	175
α7nAChR		Nicotine	↑p38 MAPK	ND	↑tau No effect on Aβ ₄₀ or Aβ ₄₂ ↓α7nAChR	154
		Nicotine	ND	ND	↓Aβ ₄₀ and Aβ ₄₂ ↓Amyloid plaque formation	186,187
		Aβ ₄₂ treatment	↑ERK1, ERK2 and JNK1	ND	↑tau	188
		Aβ ₄₂ treatment	↓ERK2 and CREB	ND	↑α7nAChR	189, 190
		Aβ ₄₂ treatment	ND		↓nAChR currents	191
		Genetic ablation (PDAPP)	ND		No effect on Aβ or plaque formation ↑synaptic markers and LTP Improved cognition	192
NMDA receptor		Memantine** or MK-801 (antagonist)	NA	Channel blocker	↓APP, sAPP, sAPPα, Aβ ₄₀ and Aβ ₄₂	190, 193–196
FPRL1		Aβ ₄₂ treatment	↑PLD, ERK1 and ERK2	ND	Internalization of Aβ	197
SSTR		Somatostatin	ND	↑Neprilysin	↓Aβ ₄₂	139

*These compounds are in Phase II trials for the treatment of AD. **This compound has US Food and Drug Administration approval for the treatment of AD. Aβ, amyloid-β; AC, adenylate cyclase; ACPD, 1-aminocyclopentane-1,3-dicarboxylic; AD, Alzheimer's disease; ADAM, a disintegrin and metalloproteinase; AMY, amyloid; APLP2, amyloid-like protein 2; A_{2A}R, adenosine 2A receptor; β₂AR, β₂ adrenergic receptor; AT₂R, angiotensin type 2 receptor; cAMP, cyclic AMP; CCL2, CC-chemokine ligand 2; CCR2, CC-chemokine receptor 2; CREB, cyclic AMP-responsive element-binding protein; CRHR1, corticotrophin-releasing hormone receptor type 1; CXCR2, CXC-chemokine receptor 2; CX₃CR1, CX₃C-chemokine receptor 1; DAG, diacyl glycerol; DHPG, dihydroxyphenylglycine; DOR, δ-opioid receptor; EPAC, exchange protein directly activated by cAMP 1; ERK, extracellular signal-regulated kinase; FPRL1, formyl peptide receptor-like 1; GLP1R, glucagon-like peptide 1 receptor; Glu, glutamate; GPR3, G protein-coupled receptor 3; GSK3β, glycogen synthase kinase 3β; 5-HT₆, 5-hydroxytryptamine receptor; JNK, Jun N-terminal kinase; NA, not applicable; LTP, long-term potentiation; mAChR, muscarinic acetylcholine receptor; MAPK, mitogen-activated protein kinase; mGluR, metabotropic glutamate receptor; nAChR, nicotinic acetylcholine receptor; ND, not determined; NF-κB, nuclear factor κB; PACAP, pituitary adenylate cyclase-activating polypeptide; PAC₁R, pituitary adenylate cyclase 1 receptor; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLA2, phospholipase A2; PLC, phospholipase C; PLD, phospholipase D; PS1, presenilin 1; sAPP, soluble amyloid precursor protein; sAPPα, soluble amino-terminal ectodomain of APP; SSTR, somatostatin receptor.

As with 5-HT_{2A}, the post-mortem brains of patients with AD display a reduction in the number of 5-HT₄ receptor binding sites in the hippocampus⁵⁸. However, it remains unclear whether the effects of the 5-HT₄ receptor on APP metabolism are directly linked to an increase in the activity of the α-secretases or an effect on cellular trafficking of APP. Nevertheless, the beneficial effects on APP processing and the enhanced cognitive performance observed *in vivo* provide a theoretical foundation for further development of 5-HT receptor-mediated AD therapeutics.

In the CNS, the 5-HT₆ receptor is mainly localized in the striatum, hippocampus and cortex in rodents⁵⁹, and predominantly in the caudate nucleus and to a lesser extent the hippocampus and amygdala in humans⁶⁰. Although the 5-HT₆ receptor has not been shown to directly modulate α-secretase activity, 5-HT₆ receptor antagonism improves cognition and memory formation and retention⁶¹. This effect is probably mediated by increasing the release of glutamate and/or ACh, which enhances memory consolidation (reviewed in REF. 62).

5-HT₆ receptor antagonists have met with some success in Phase I and Phase II clinical trials. Cognitive improvement was observed in patients with AD treated with SB-742457 following completion of a Phase II clinical trial. SAM-531, another 5-HT₆ receptor antagonist, has also completed an initial Phase II clinical trial with some success (reviewed in REFS 63,64). Interestingly, a polymorphism in the 5-HT₆ gene seems to be a risk factor for AD, as patients with AD are more likely to carry the C267T allele variant⁶⁵, and 5-HT₆ receptor expression is decreased in the prefrontal cortex of patients with AD⁶⁶.

Corticotrophin-releasing hormone receptor type I. Activation of the corticotrophin-releasing hormone (CRH) receptor type I (CRHR1) by CRH stimulates an increase in the release of sAPP α in rat cerebellar neurons, and to a lesser extent in the human neuroblastoma IMR32 cell line and in mouse hippocampal HT22 cells⁶⁷, although it remains to be determined whether this is mediated by α -secretase. Brain areas affected in AD show morphological abnormalities in CRH-containing neurons and also a dramatic reduction in the CRH levels^{68,69}. Moreover, cognitive impairment is accompanied by decreased concentrations of CRH in the cerebrospinal

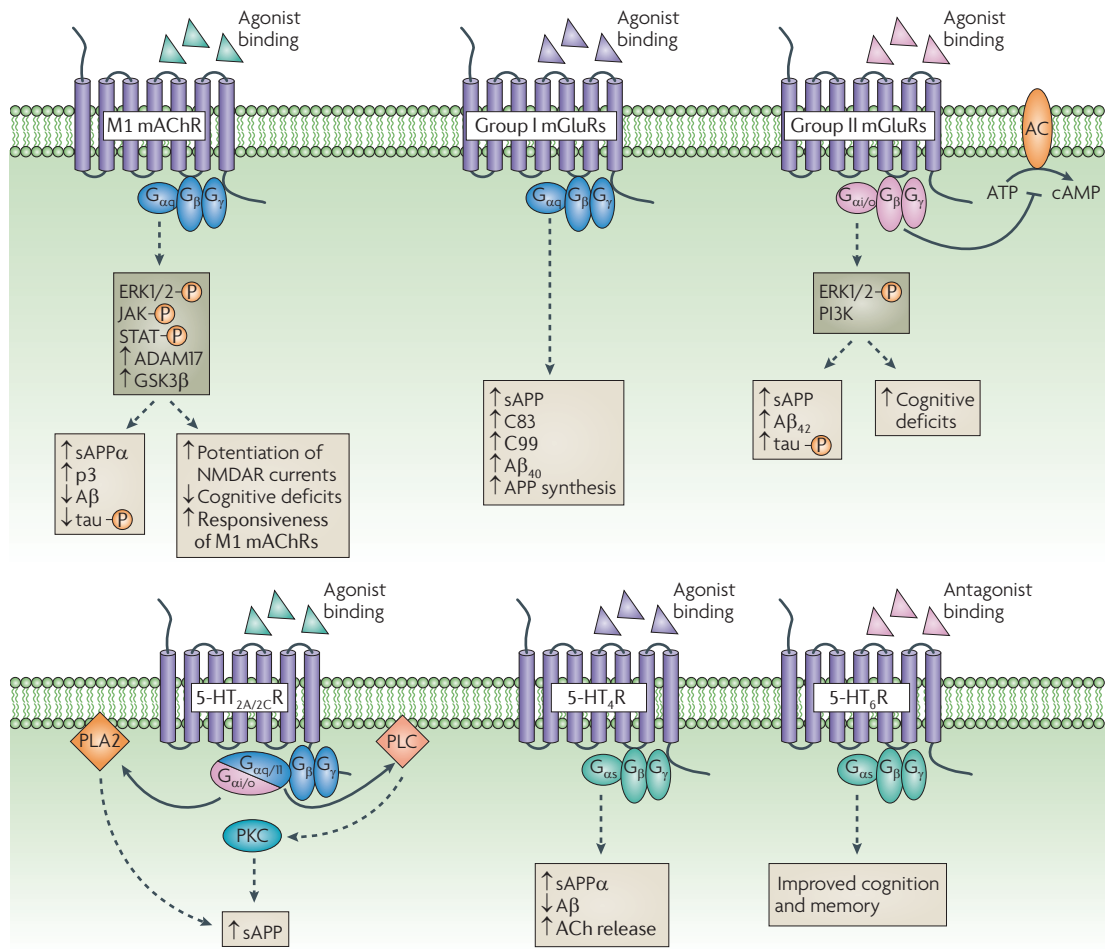


Figure 2 | GPCR signalling and the α -secretase pathway. G protein-coupled receptors (GPCRs) exert their multiple functions through a complex network of intracellular signalling pathways. Ligand-bound GPCRs activate heterotrimeric G proteins, inducing the exchange of GDP for GTP and the formation of a GTP-bound G α subunit and the release of a G $\beta\gamma$ dimer. The G protein subunits then activate specific secondary effector molecules, such as adenylyl cyclase (AC), phospholipase C (PLC) and phospholipase A2 (PLA2), leading to the generation of secondary messengers and activation of extracellular signal-regulated kinase 1/2 (ERK1/2), Janus kinase (JAK) and phosphoinositide 3-kinase (PI3K), and modulation of the α -secretase pathway. In the case of the M1 muscarinic acetylcholine receptor (M1 mAChR), the group I metabotropic glutamate receptors (mGluRs) and the 5-hydroxytryptamine receptors 5-HT_{2A/2C}-R and 5-HT₄-R, agonist stimulation leads to an increase in soluble amyloid precursor protein (sAPP) release, a decrease in amyloid- β (A β) generation, a decrease in tau phosphorylation and/or an alleviation of the cognitive deficits in a mouse model of Alzheimer's disease (AD). Conversely, agonist stimulation of the Group II mGluRs leads to an increase in amyloid- β ₄₂ generation, tau phosphorylation and an exacerbation of the cognitive deficits in an AD mouse model. In the case of the 5-HT₆ receptor (5-HT₆-R), antagonism of the receptor leads to an improvement in cognition. Solid arrows represent direct signalling pathways and dashed arrows represent signalling via intermediates that are not shown. ACh, acetylcholine; ADAM, a disintegrin and metalloproteinase; cAMP, cyclic AMP; GSK3 β , glycogen synthase kinase 3 β ; NMDAR, NMDA receptor; PKC, protein kinase C; sAPP α , soluble amino-terminal ectodomain of APP; STAT, signal transducer and activator of transcription.

fluid⁷⁰. A possible application of CRH for the treatment of AD is mainly based on the memory-enhancing effects of CRH in rodents⁷¹.

Pituitary adenylate cyclase 1 receptor. The pituitary adenylate cyclase 1 receptor (PAC₁R) is a GPCR that is stimulated by the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP). The receptor is primarily localized to the hypothalamus but is also expressed in the cerebral cortex and hippocampus⁷², areas of the human brain affected by AD. The major form of PACAP, composed of 38 amino acids (PACAP38), has been shown to improve memory in rats⁷³. Together with a C-terminal truncated form, PACAP27, it stimulates an increase in sAPP α release⁷⁴. This effect is blocked by a broad-spectrum metalloprotease inhibitor and by an ADAM10-specific inhibitor, GI254023X⁷⁴. Thus, stimulation of PAC₁R enhances α -secretase activity. Although the molecular mechanism of this effect has not been elucidated, neuropeptide hormones such as PACAP27 and PACAP38 display a high flux rate across the blood–brain barrier (BBB)⁷⁵, which should permit the *in vivo* examination of the effect of PACAP in a transgenic mouse model of AD.

Regulation of β -secretase

The β -secretase BACE1 (β -site APP-converting enzyme 1), is a type I transmembrane aspartyl protease that is active at low pH and is predominantly localized in acidic intracellular compartments, such as endosomes and the *trans*-Golgi network. Cleavage of APP by BACE1 generates a soluble N-terminal ectodomain of APP (sAPP β) and the N terminus of amyloid- β . Subsequent cleavage of the membrane-bound C-terminal fragment C99 by the γ -secretase liberates the amyloid- β peptide species (FIG. 1).

BACE1 is abundantly expressed in neurons in the brain. *Bace1*^{-/-} mice are viable and fertile, facilitating the study of the role of this enzyme in AD. BACE1 deficiency in an AD mouse model abrogates amyloid- β generation, amyloid pathology, electrophysiological dysfunction and cognitive deficits, implying that therapeutic inhibition of BACE1 would decrease generation of all amyloid- β species. However, *Bace1*^{-/-} mice display phenotypic abnormalities that are related to the processing of additional proteins by BACE1, suggesting that therapeutic inhibition of BACE1 could have adverse side effects (reviewed in REFS 76,77). Nevertheless, BACE1 is arguably the primary therapeutic target to deter amyloid- β generation. Detailed structural analysis of BACE1 has led to the discovery of many transition state-based inhibitors with activity in the low nanomolar range, although the *in vivo* efficacy of these compounds is limited because most of them do not penetrate the BBB or are actively exported from the brain by P-glycoprotein. Recent evidence suggests that GPCRs such as the δ -opioid receptor (DOR)⁷⁸ could provide a therapeutic opportunity to modulate BACE1 and amyloid- β generation.

δ - and μ -opioid receptors. The opioid receptors, which play important parts in learning and memory, are deregulated in specific regions of the AD brain⁷⁹. There

is evidence to suggest that the DOR, together with the β_2 adrenergic receptor (β_2 -AR), promotes the γ -secretase-mediated cleavage of the APP C-terminal fragment after its generation by β -secretase⁸⁰. A more recent study by the same group suggested that activation of the DOR promotes the translocation of a complex consisting of the DOR, β -secretase and γ -secretase from the cell surface to the late endosomes and lysosomes (LEL), which results in enhanced β - and γ -secretase proteolysis of APP⁷⁸. In a mouse model of AD, administration of natriindole, a selective DOR antagonist, improved spatial learning and reference memory, and reduced the amyloid plaque burden⁷⁸. Similarly, *in vivo* knock down of the DOR reduced amyloid- β_{40} accumulation in the hippocampus of an AD mouse model. However, there was no effect on the more hydrophobic (and therefore more toxic) amyloid- β_{42} (REF. 78). By contrast, administration of a μ -opioid receptor (MOR) antagonist had no effect on amyloid- β generation or amyloid plaque formation and was unable to reverse the learning and memory deficiency of the AD mouse model⁷⁸, although another group reported improved spatial memory retention in this transgenic AD mouse model⁸¹.

DOR binding is decreased in the amygdala and ventral putamen, and MOR binding is decreased in the hippocampus and subiculum⁷⁹ of post-mortem brain samples from patients with AD. Elevated hippocampal levels of enkephalin, the ligand for these receptors, have been detected in AD transgenic mice and in the human AD brain^{81,82}. Excessive stimulation by enkephalin may uncouple the opioid receptors from G proteins, resulting in receptor internalization^{83,84} and reduced receptor binding in patients with AD^{79,85}. These adaptive changes in opioid receptor expression in response to increased enkephalin levels might limit the efficacy of opioid receptor antagonists in AD and could explain the variable effects of different DOR antagonists on amyloid- β generation in AD transgenic mouse models.

Regulation of γ -secretase

The γ -secretase complex is composed of four integral membrane proteins: the catalytic component presenilin 1 (PS1) or PS2 and the essential cofactors nicastrin, anterior pharynx defective 1 (APH1) and presenilin enhancer 2 (PEN2)⁸⁶. Proteolysis of the α -cleavage product C83 by the γ -secretase complex generates a short p3 fragment, which precludes formation of amyloid- β . By contrast, proteolysis of the β -secretase product C99 by the γ -secretase complex generates the amyloid- β peptide, which ranges in length from 35 to 43 residues (FIG. 1). The majority of amyloid- β produced is 40 amino acids in length (amyloid- β_{40}), whereas a small proportion (~10%) is the 42-residue variant (amyloid- β_{42}). Several γ -secretase inhibitors have been developed but they have limited clinical efficacy owing to the severe side effects associated with inhibition of the Notch receptor, which is a substrate for γ -secretase proteolysis. Therefore, determination of the cellular mechanisms that specifically regulate amyloid- β generation by γ -secretase is of crucial importance for understanding the factors that cause AD and could highlight new therapeutic targets.

β_2 -adrenergic receptor. Stimulation of β_2 -AR increases amyloid- β generation *in vitro*, independently of an elevation in cAMP levels⁸⁰. In an AD transgenic mouse model, treatment with a β_2 -AR agonist or antagonist respectively increased and decreased the amyloid plaque burden⁸⁰. It has been suggested that the β_2 -AR constitutively associates with PS1 at the plasma membrane and undergoes clathrin-mediated endocytosis together with the γ -secretase complex following agonist stimulation⁸⁰. This proposed localization of the γ -secretase in LEL compartments, which is supported by other studies^{87,88}, could promote cleavage of C99 and thereby the generation of amyloid- β ⁸⁰. As a therapeutic application, it will be important to determine whether β_2 -AR activation also modulates cleavage of the Notch receptor, given the adverse side effects of targeting γ -secretase discussed above. Importantly, the β_2 -AR is expressed in the hippocampus and the cortex in humans⁸⁹, and polymorphisms in the gene encoding the β_2 -AR are associated with an increased risk of developing sporadic late-onset AD⁹⁰, providing support for the potential clinical relevance of the *in vitro* and AD mouse model findings.

G protein-coupled receptor 3. G protein-coupled receptor 3 (GPR3) is an orphan GPCR with a putative ligand⁹¹ that has not been validated^{92,93}. The receptor was identified as a modulator of amyloid- β generation in a high-throughput functional genomics screen designed to identify potential therapeutic targets for AD⁹². GPR3 is strongly expressed in neurons in the hippocampus, amygdala, cortex, entorhinal cortex and thalamus in the normal human brain^{94,95}, and its expression is increased in a subset of patients with sporadic AD⁹².

Several lines of evidence support the involvement of GPR3 in the generation of amyloid- β . *In vitro* models of AD suggest that this effect is independent of its ability to stimulate the production of cAMP⁹². In an AD transgenic mouse model⁹⁶, hippocampal overexpression of GPR3 enhanced amyloid- β_{40} and amyloid- β_{42} generation in the absence of an effect on γ -secretase expression⁹². Genetic ablation of *Gpr3* in these mice dramatically reduced amyloid- β_{40} and amyloid- β_{42} levels⁹², demonstrating that endogenous GPR3 is involved in amyloid- β generation. Further *in vitro* studies suggested that GPR3 promotes increased association of the individual γ -secretase complex components within detergent-resistant membrane domains and stabilizes the mature γ -secretase complex⁹².

Thus, similar to the β_2 -AR, the effect of GPR3 signalling on amyloid- β generation is not mediated through an elevation in cAMP levels. Rather, both GPCRs modulate the trafficking and/or localization of the γ -secretase complex to membrane domains where it can more efficiently process the β -secretase product C99. Importantly, the *in vitro* effect of GPR3 expression on amyloid- β generation occurs in the absence of an effect on Notch processing, suggesting that GPR3 can selectively target specific γ -secretase pathways.

CXC-chemokine receptor 2. The CXC-chemokine receptor type 2 (CXCR2) is abundantly expressed in neurons and is strongly upregulated in a subpopulation of neuritic

plaques in the post-mortem human AD brain^{97,98}. In an AD transgenic mouse model, treatment with the CXCR2 antagonist SB-225002 reduces amyloid- β_{40} levels⁹⁹ and is accompanied by a reduction in PS1-C-terminal fragment (CTF) levels, resulting in a probable decrease in the proteolytically active mature γ -secretase complex⁹⁹. Crossing the *Cxcr2*-deficient mouse with an AD transgenic mouse also results in a decrease in amyloid- β_{40} and amyloid- β_{42} generation, and γ -secretase complex expression¹⁰⁰. *In vitro* evidence suggests that antagonism of CXCR2 reduces expression levels of other γ -secretase complex components, inhibiting generation of both the AICD and the Notch intracellular domain. Whether CXCR2 is involved in enhanced turnover, degradation or stabilization of the PS1-CTF has not been determined. However, inhibition of Jun N-terminal kinase (JNK) activity, which is involved in signalling downstream of CXCR2, correlates with reduced phosphorylation and stability of the PS1-CTF^{101,102}. Given that antagonism of CXCR2 leads to general changes in γ -secretase expression and activity, it will be challenging to therapeutically target CXCR2.

GPCRs and amyloid- β toxicity

One of the most puzzling aspects of the amyloid cascade hypothesis is why amyloid- β exerts a neurotoxic effect on cells. There is no clear correlation between exposure of the brain to amyloid- β plaques and neurodegeneration and, in cell culture models, the toxicity associated with amyloid- β is variable and poorly understood. Small oligomeric structures of amyloid- β , known as amyloid- β -derived diffusible ligands (ADDLs)¹⁰³, cause synaptotoxicity, interfering with glutamate signalling at several levels, including direct and indirect effects on Ca^{2+} levels, endocytosis, and possibly membrane damage and clustering of various membrane proteins. A further complication is that a component of the toxicity associated with amyloid- β might be the consequence of a general mechanism such as interaction with the plasma membrane, which could affect multiple GPCRs. Moreover, several GPCRs are involved in neuroinflammation, with beneficial or detrimental effects on amyloid- β -mediated toxicity depending on the model under investigation.

Thus, it remains unclear how the involvement of GPCRs in amyloid- β -mediated toxicity can be clinically exploited. Studies on the angiotensin type 2 receptor (AT_2R), the adenosine A_{2A} receptor (A_{2A}R) and CC-chemokine receptor 2 (CCR2) provide insight into this complicated matter.

Angiotensin type 2 receptor. Angiotensin II and the AT_2R have been implicated in several CNS functions, including neuronal apoptosis¹⁰⁴, behaviour¹⁰⁵ and memory¹⁰⁶. Activation of AT_2R inhibits stimulation of $\text{G}\alpha_{i/o}$ and $\text{G}\alpha_{q/11}$ by the AT_1 receptor¹⁰⁷. Interestingly, constitutive and muscarinic agonist-dependent $\text{G}\alpha_{q/11}$ signalling is also impaired in the AD brain^{108,109}. There is evidence that oxidative stress, induced by elevated amyloid- β levels, leads to dimerization of AT_2R ¹¹⁰. Further elevation in amyloid- β levels initiates oligomerization of AT_2R dimers and sequestration of

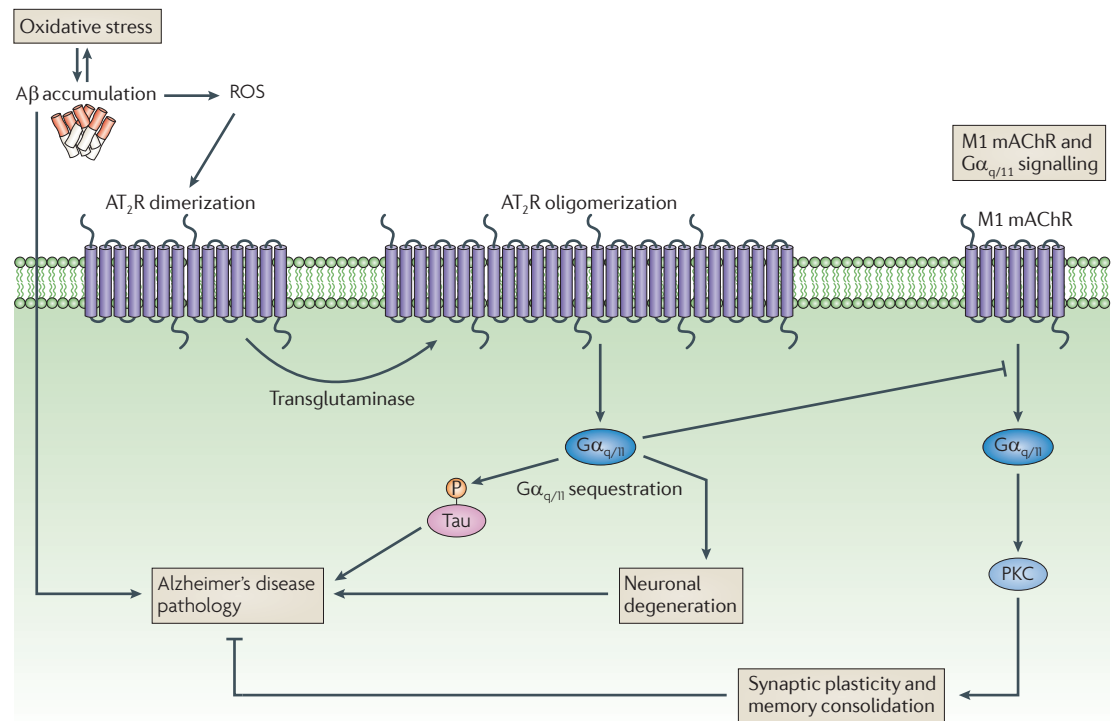


Figure 3 | Amyloid- β toxicity and deregulation of AT_2R and M1 mAChR signalling. Oxidative stress and amyloid- β ($A\beta$) accumulation leads to an increase in reactive oxygen species (ROS) generation and dimerization of angiotensin type 2 receptors (AT_2R). An increase in levels of the protein-crosslinking enzyme transglutaminase, as occurs in Alzheimer's disease, and further $A\beta$ deposition trigger crosslinking and subsequent oligomerization of AT_2R dimers. The AT_2R oligomers sequester $G\alpha_{q/11}$ and thereby inhibit $G\alpha_{q/11}$ from coupling to M1 muscarinic acetylcholine receptors (M1 mAChRs). Sequestration of $G\alpha_{q/11}$ results in tau phosphorylation, neuronal degeneration and Alzheimer's disease progression. PKC, protein kinase C. Figure is reproduced, with permission, from REF. 111 © (2009) American Association for the Advancement of Science.

$G\alpha_{q/11}$ by AT_2R oligomers. The subsequent decrease in M1 mAChR- $G\alpha_{q/11}$ coupling and activation correlates with hippocampal neurodegeneration, tau phosphorylation and neuronal loss, contributing to the development and exacerbation of the neuropathology of AD (reviewed in REF. 111) (FIG. 3). This study indicates that stimulation of M1 mAChR signalling, which alleviates the aetiopathology of AD (discussed above), might not be sufficient to improve cognition in patients owing to concomitant toxic effects of the accumulation of amyloid- β on other receptors and their downstream signalling pathways.

Adenosine A_{2A} receptor. The adenosine receptors are GPCRs that are classified as A_1 , A_{2A} , A_{2B} and A_3 receptors. A_1 and A_3 receptors inhibit adenylyl cyclase through $G_{i/o}$ proteins, whereas A_{2A} and A_{2B} receptors stimulate adenylyl cyclase through G_s proteins¹¹². The A_{2A} Rs are mainly expressed in striatal neurons in the human brain. In patients with AD, they are also abundantly expressed in microglia, the hippocampus and the cortex¹¹³. Expression of the A_1 and A_{2A} receptors is elevated in the frontal cortex of post-mortem brains of patients with AD¹¹⁴. A_{2A} R-deficient mice display improved spatial recognition memory¹¹⁵, whereas *in vivo* overexpression of the A_{2A} R leads to memory deficits¹¹⁶. Consequently, pharmacological blockade

or gene disruption of adenosine A_{2A} Rs confers neuroprotection (FIG. 4a). Furthermore, caffeine, an A_1 and A_{2A} receptor antagonist, is neuroprotective against amyloid- β -induced neurotoxicity in cultured neurons of rats¹¹⁷ and against amyloid- β -induced cognitive impairments in mice¹¹⁸ (FIG. 4b). Long-term administration of caffeine to APP transgenic mice also improves cognition and reduces amyloid- β_{40} and amyloid- β_{42} generation, and is accompanied by a modest reduction in PS1 and BACE1 protein expression levels¹¹⁹. This is supported by studies in rats using a selective A_{2A} R antagonist, SCH 58261, which is protective against amyloid- β_{42} -induced synaptotoxicity and memory impairment^{118,120}. However, earlier studies have suggested that caffeine, via ryanodine receptor-regulated intracellular calcium release channels, stimulates an increase in amyloid- β generation *in vitro*¹²¹. Nevertheless, caffeine also stimulates acetylcholine release, an effect that is mediated by blockade of the A_{1A} R^{122,123}. Collectively, these findings suggest that the adenosinergic system is a promising therapeutic avenue for the management of the cognitive dysfunction in AD.

Chemokine receptors. Microglia are macrophages of the CNS. They have been shown to surround amyloid plaques both in patients with AD and in AD transgenic mouse models¹²⁴, but whether they have

beneficial or detrimental effects on plaque formation remains unclear. Chemokine receptors are GPCRs that are expressed by microglia. CCR2 is required for macrophage infiltration at sites of axonal injury in the hippocampus¹²⁵. The main ligand for CCR2, CC-chemokine ligand 2 (CCL2; also known as MCP1), has been localized to mature amyloid plaques in the AD brain¹²⁶. Stimulation of microglia and astrocytes with amyloid- β leads to an elevation in CCL2 levels¹²⁷. In a mouse model of AD, *Ccr2* deficiency correlates with decreased microglial accumulation and increased amyloid- β deposition¹²⁸.

A recent study suggests the chemokine receptor CX₃C-chemokine receptor 1 (CX₃CR1) plays a part in the recruitment of microglia to injured neurons^{129,130} and is involved in neuronal loss¹³¹. The effect on amyloid plaque load was not assessed in this AD mouse model. Nevertheless, a reduction in — or almost complete ablation of — microglia does not affect the amyloid plaque load¹³² in another AD mouse model, suggesting that microglia are not essential for initiation of cerebral amyloidosis.

GPCRs and amyloid- β degradation

Promoting amyloid- β clearance from the brain is an alternative therapeutic strategy to inhibition of amyloid- β generation. Such an approach is the basis for the passive and active immunotherapy with amyloid- β -specific antibodies. However, stimulation of GPCRs, in particular the somatostatin receptor, could represent an interesting alternative approach to promoting amyloid- β clearance, as these GPCRs induce expression of amyloid- β -degrading enzymes, such as neprilysin, in the brain. A combination of memory enhancement, neuroprotection and anti-amyloid- β activity makes this an attractive therapeutic approach for AD.

Somatostatin receptors. Somatostatin (also known as somatotropin release-inhibiting factor, SRIF) is a regulatory peptide with two bioactive forms, SRIF14 and SRIF28, which are widely expressed throughout the CNS and function in neurotransmission, protein secretion and cell proliferation^{133,134}.

Expression of the two most abundant SRIF receptors in the brain, somatostatin receptor type 2 (SSTR2) and SSTR4, is reduced in the cortex of human patients with AD¹³⁵. Interestingly, intracerebroventricular injection of amyloid- β _{25–35} results in a selective decrease in SSTR2 mRNA and protein levels in the temporal cortex of rats, whereas cognitive deficits correlate with reduced SRIF concentrations in the CSF¹³⁶ or middle front gyrus (Brodmann area 9)¹³⁷. SRIF levels are also reduced in the CSF¹³⁶, cortex¹³⁵ and hippocampus¹³⁸ of patients with AD.

Compelling evidence suggests that SRIF is a modulator of neprilysin activity in the brain¹³⁹. Neprilysin, one of the main amyloid- β -degrading enzymes, regulates the steady state levels of amyloid- β ₄₀ and amyloid- β ₄₂ *in vivo*¹⁴⁰. SRIF has been shown to significantly elevate neprilysin levels in primary murine cortical neuronal cultures, which accompanies a reduction in amyloid- β ₄₂ levels¹³⁹. Conversely, neprilysin activity and localization are altered in the hippocampus of SRIF-deficient mice, with a corresponding increase in amyloid- β ₄₂ levels¹³⁹. There are conflicting results from AD transgenic mouse models, which show either an increase¹⁴¹ or a decrease in SRIF levels¹⁴². Further work is necessary to clarify the cause of the changes in SRIF levels in these AD models.

Additional modulators of APP metabolism

This Review highlights the role of GPCRs and their signal transduction cascades in the metabolism of APP. However, numerous additional effectors and

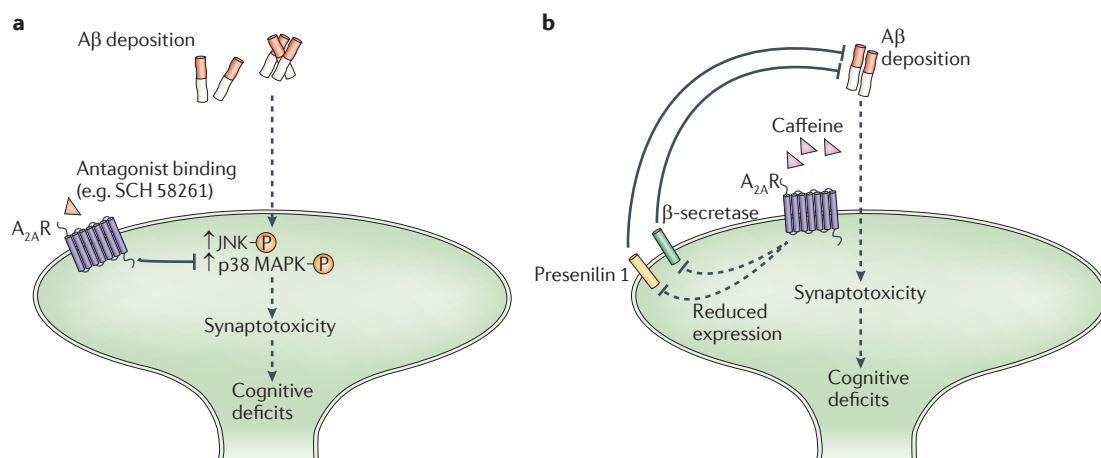


Figure 4 | Adenosine A_{2A} receptor and amyloid- β -mediated toxicity. **a** | Amyloid- β (A β) deposition has been shown to activate the p38 mitogen-activated protein kinase (MAPK) signalling pathway, which leads to A β -induced neurotoxicity. Pharmacological blockade of the adenosine A_{2A} receptor (A_{2A}R) with the compound SCH 58261 reduces A β -induced p38 MAPK phosphorylation, synaptotoxicity and cognitive impairment. **b** | Similarly, caffeine, an A_{2A}R antagonist, is also protective against A β -mediated toxicity and may regulate the expression levels of the β -secretase, via the cRaf-1/nuclear factor- κ B pathway and presenilin 1, which leads to a decrease in A β ₄₀ and A β ₄₂ deposition and is protective against cognitive impairment in an Alzheimer's disease mouse model. Solid arrows represent direct signalling pathways and dashed arrows represent signalling via intermediates that are not shown. JNK, Jun N-terminal kinase.

Box 2 | GPCRs, diabetes and Alzheimer's disease

Glucagon-like peptide 1 receptor

Type 2 diabetes (T2D) has been identified as a risk factor for Alzheimer's disease (AD)¹⁵⁵, and insulin signalling has a role in learning and memory¹⁵⁶⁻¹⁵⁸, which potentially links insulin resistance to AD dementia. Indeed, deregulated insulin signalling has been observed in brains of patients with AD and may contribute to the development of AD¹⁵⁹. The combination of insulin with other antidiabetic medications is also associated with lower amyloid plaque density and a diminution of the cognitive decline associated with AD^{160,161}.

Strategies have therefore been developed to normalize insulin signalling in the brain to deter the progression of AD¹⁶². One promising intervention is the use of the incretin hormone glucagon-like peptide 1 (GLP1) as a treatment for neurodegenerative diseases¹⁶³. *In vivo* administration of GLP1 or exendin-4, a more stable analogue of GLP1, reduces endogenous levels of amyloid- β_{40} in the mouse brain and protects against cell death¹⁶⁴. In addition, GLP1 and the stable analogue (Val⁸)GLP1 enhance long-term potentiation (LTP) and reverse the LTP impairment induced by amyloid- β_{25-35} administration in rodents, which might underlie an improvement in cognitive function¹⁶⁵. Most recently, (Val⁸)GLP1 also prevented amyloid- β_{40} -induced impairment in late-phase LTP, and spatial learning and memory in rodents¹⁶⁶. Some evidence also suggests that the desensitization of insulin receptors that occurs in AD can be reversed by activation of GLP1 receptors (GLP1Rs)¹⁶⁷.

GLP1 binds to GLP1R, which activates diverse signalling pathways, including cyclic AMP, protein kinase A, phospholipase C, phosphatidylinositol 3-kinase, protein kinase C and mitogen-activated protein kinase¹⁶⁸⁻¹⁷¹. GLP1R-deficient mice display an impairment in synaptic plasticity¹⁶³ and a decrease in the acquisition of contextual learning, a learning deficit that can be reversed following hippocampal gene transfer of *Glp1r*¹⁷². By contrast, overexpression of GLP1R through hippocampal gene transfer markedly enhanced learning and memory in rodents¹⁷². Taken together, these studies suggest that the GLP1R represents a novel and promising therapeutic target for AD.

Amylin receptor

Amylin (also known as islet amyloid polypeptide) is a peptide that was first isolated from amyloid deposits from the pancreatic islets of Langerhans of patients with type 2 diabetes¹⁷³. Interestingly, human amylin, which acts through the G protein-coupled amylin receptor, possesses amyloidogenic and neurotoxic properties similar to amyloid- β ¹⁷⁴. Accordingly, treatment of rat neuronal cultures with an amylin receptor antagonist, AC187, attenuates amyloid- β_{42} - and amylin-induced neurotoxicity by blocking caspase activation¹⁷⁵. It would be interesting to determine whether treatment with GLP1 could alleviate the cognitive deficits, and to determine the expression levels of GLP1R in this diabetic AD mouse model.

Most recently, studies conducted by crossing two T2D mouse models with an AD mouse model have provided further mechanistic insight into the relationship between diabetes and AD, demonstrating that the onset of diabetes exacerbates cognitive dysfunction in the absence of an elevation in amyloid- β levels and leads to increased cerebrovascular inflammation and amyloid angiopathy¹⁷⁶. Conversely, the diabetic AD mice display an accelerated diabetic phenotype relative to the diabetic mouse model alone, suggesting that the amyloid pathology may adversely affect the T2D and vice versa.

non-GPCRs have been implicated as modulators of APP catabolism and metabolism. These studies have been thoroughly addressed in a review by Slack and Wurtman¹⁴³. Briefly, growth factors such as nerve growth factor — which signals through neurotrophic tyrosine kinase receptor type 1 (NTRK1) and nerve growth factor receptor (NGFR; also known as p75NTR), a member of the tumour necrosis factor receptor family — as well as epidermal growth factor and fibroblast growth factor have been shown to enhance APP synthesis and sAPP secretion. In addition, several inflammatory cytokines are elevated in the serum and/or brains of patients with AD, and various cytokines, interleukins and prostaglandin E2 have also been implicated in the modulation of APP synthesis, sAPP secretion and/or amyloid- β deposition. Besides neurotransmitters, growth factors and cytokines, the hormone signalling molecules oestrogen and testosterone, vasopressin and bradykinin have also been implicated in the regulation of APP synthesis and metabolism.

Concluding remarks

Numerous drug discovery efforts target the inhibition of amyloid- β production, the prevention of amyloid- β aggregation and the enhancement of amyloid- β clearance.

Although these may seem to be straightforward biochemical pathways, several feedback loops enhance not only amyloid- β deposition but also its toxicity, clearance and overall impact on memory function and neuronal health. Such feedback loops also imply that a monotherapy will not be sufficient to prevent the progression of AD. Based on the discussion above, it is clear that several GPCRs are involved at many stages of AD disease progression (TABLE 1). There also seems to be a pathologically reinforcing loop between type 2 diabetes and AD, with GPCRs providing an avenue for therapeutic intervention for both diseases (BOX 2). Drugs that target GPCRs could diversify the symptomatic therapeutic portfolio for AD and potentially provide disease-modifying treatments. In this sense, they complement the current areas of investigation, which are primarily focused on secretase inhibitors⁷⁷ and amyloid immunotherapy¹⁴⁴.

Given that the current anti-amyloidogenic therapy under development is considered to be most effective as a preventative measure or in early stages of AD, additional drugs that preferentially enhance cognition will become a necessary complement to treatment, especially as the disease progresses to more advanced stages. In this regard, GPCRs represent the largest therapeutic

target in the pharmaceutical industry and provide ample opportunities for AD-related drug development. Nevertheless, progress in the field is hampered by the difficulty in developing highly receptor-specific ligands and the adverse side effects of currently available drugs. Recent advances in the GPCR field suggest

that a more functional approach towards the classification of GPCRs, which are now organized according to structural similarity, might enhance the therapeutic potential of GPCRs and assist in the development of selective GPCR candidate drugs for AD and many other diseases.

1. De Strooper, B. Proteases and proteolysis in Alzheimer disease: a multifactorial view on the disease process. *Physiol. Rev.* **90**, 465–494 (2010).
2. Furukawa, K. *et al.* Increased activity-regulating and neuroprotective efficacy of alpha-secretase-derived secreted amyloid precursor protein conferred by a C-terminal heparin-binding domain. *J. Neurochem.* **67**, 1882–1896 (1996).
3. Small, D. H. *et al.* A heparin-binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. *J. Neurosci.* **14**, 2117–2127 (1994).
4. Ishida, A., Furukawa, K., Keller, J. N. & Mattson, M. P. Secreted form of beta-amyloid precursor protein shifts the frequency dependency for induction of LTD, and enhances LTP in hippocampal slices. *Neuroreport* **8**, 2133–2137 (1997).
5. Sennvik, K. *et al.* Levels of alpha- and beta-secretase cleaved amyloid precursor protein in the cerebrospinal fluid of Alzheimer's disease patients. *Neurosci. Lett.* **278**, 169–172 (2000).
6. Jang, H. *et al.* Truncated beta-amyloid peptide channels provide an alternative mechanism for Alzheimer's Disease and Down syndrome. *Proc. Natl Acad. Sci. USA* **107**, 6538–6543 (2010).
7. Buxbaum, J. D. *et al.* Processing of Alzheimer beta/A4 amyloid precursor protein: modulation by agents that regulate protein phosphorylation. *Proc. Natl Acad. Sci. USA* **87**, 6003–6006 (1990).
This is the first study to demonstrate that proteolytic processing of APP involves a signal transduction cascade via activation of PKC.
8. Caporaso, G. L., Gandy, S. E., Buxbaum, J. D., Ramabhadran, T. V. & Greengard, P. Protein phosphorylation regulates secretion of Alzheimer beta/A4 amyloid precursor protein. *Proc. Natl Acad. Sci. USA* **89**, 3055–3059 (1992).
9. Efthimiopoulos, S. *et al.* Intracellular cyclic AMP inhibits constitutive and phorbol ester-stimulated secretory cleavage of amyloid precursor protein. *J. Neurochem.* **67**, 872–875 (1996).
10. Robert, S. J., Zugaza, J. L., Fischmeister, R., Gardier, A. M. & Lezoualc'h, F. The human serotonin 5-HT₄ receptor regulates secretion of non-amyloidogenic precursor protein. *J. Biol. Chem.* **276**, 44881–44888 (2001).
11. Xu, H., Sweeney, D., Greengard, P. & Gandy, S. Metabolism of Alzheimer beta-amyloid precursor protein: regulation by protein kinase A in intact cells and in a cell-free system. *Proc. Natl Acad. Sci. USA* **93**, 4081–4084 (1996).
12. Mills, J. *et al.* Regulation of amyloid precursor protein catabolism involves the mitogen-activated protein kinase signal transduction pathway. *J. Neurosci.* **17**, 9415–9422 (1997).
13. Solano, D. C. *et al.* Insulin regulates soluble amyloid precursor protein release via phosphatidylinositol 3 kinase-dependent pathway. *FASEB J.* **14**, 1015–1022 (2000).
14. Buxbaum, J. D., Koo, E. H. & Greengard, P. Protein phosphorylation inhibits production of Alzheimer amyloid beta/A4 peptide. *Proc. Natl Acad. Sci. USA* **90**, 9195–9198 (1993).
15. Hung, A. Y. *et al.* Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. *J. Biol. Chem.* **268**, 22959–22962 (1993).
16. da Cruz e Silva, O. A. *et al.* Enhanced generation of Alzheimer's amyloid-beta following chronic exposure to phorbol ester correlates with differential effects on alpha and epsilon isozymes of protein kinase C. *J. Neurochem.* **108**, 319–330 (2009).
17. Levey, A. I., Kitt, C. A., Simonds, W. F., Price, D. L. & Brann, M. R. Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J. Neurosci.* **11**, 3218–3226 (1991).
18. Wei, J., Walton, E. A., Milici, A. & Buccafusco, J. J. m1-m5 muscarinic receptor distribution in rat CNS by RT-PCR and HPLC. *J. Neurochem.* **63**, 815–821 (1994).
19. Wess, J., Eglén, R. M. & Gautam, D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. *Nature Rev. Drug Discov.* **6**, 721–733 (2007).
20. Nitsch, R. M., Slack, B. E., Wurtman, R. J. & Growdon, J. H. Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* **258**, 304–307 (1992).
This study and reference 21 were the first to demonstrate the effect of neurotransmitter receptor activation on the proteolysis of APP. Stimulation of the M1 mAChR and the M3 mAChR increases the PKC-mediated release of sAPP.
21. Buxbaum, J. D. *et al.* Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. *Proc. Natl Acad. Sci. USA* **89**, 10075–10078 (1992).
Along with reference 20, this report demonstrates that activation of the M1 mAChR stimulates the release of sAPP.
22. Flynn, D. D., Ferrari-DiLeo, G., Mash, D. C. & Levey, A. I. Differential regulation of molecular subtypes of muscarinic receptors in Alzheimer's disease. *J. Neurochem.* **64**, 1888–1891 (1995).
23. Anagnostaras, S. G. *et al.* Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. *Nature Neurosci.* **6**, 51–58 (2003).
24. Levey, A. I. Muscarinic acetylcholine receptor expression in memory circuits: implications for treatment of Alzheimer disease. *Proc. Natl Acad. Sci. USA* **93**, 13541–13546 (1996).
25. Messer, W. S. Jr, Bohnett, M. & Stibbe, J. Evidence for a preferential involvement of M1 muscarinic receptors in representational memory. *Neurosci. Lett.* **116**, 184–189 (1990).
26. Wall, S. J. *et al.* Production of antisera selective for m1 muscarinic receptors using fusion proteins: distribution of m1 receptors in rat brain. *Mol. Pharmacol.* **39**, 643–649 (1991).
27. Caccamo, A. *et al.* M1 receptors play a central role in modulating AD-like pathology in transgenic mice. *Neuron* **49**, 671–682 (2006).
This study demonstrates that the selective M1 mAChR agonist AF267B reduces the cellular and learning and memory impairments in an AD mouse model. It also demonstrates that the underlying mechanism involves activation of ADAM17.
28. Davis, A. A., Fritz, J. J., Wess, J., Lah, J. J. & Levey, A. I. Deletion of M1 muscarinic acetylcholine receptors increases amyloid pathology *in vitro* and *in vivo*. *J. Neurosci.* **30**, 4190–4196 (2010).
29. Jones, C. K. *et al.* Novel selective allosteric activator of the M1 muscarinic acetylcholine receptor regulates amyloid processing and produces antipsychotic-like activity in rats. *J. Neurosci.* **28**, 10422–10433 (2008).
This study identifies the first specific allosteric M1 mAChR agonist, TBPD, which increases the non-amyloidogenic processing of APP and decreases amyloid-β generation.
30. Farber, S. A., Nitsch, R. M., Schulz, J. G. & Wurtman, R. J. Regulated secretion of beta-amyloid precursor protein in rat brain. *J. Neurosci.* **15**, 7442–7451 (1995).
31. Lee, R. K., Wurtman, R. J., Cox, A. J. & Nitsch, R. M. Amyloid precursor protein processing is stimulated by metabotropic glutamate receptors. *Proc. Natl Acad. Sci. USA* **92**, 8083–8087 (1995).
This is the first study to demonstrate that the metabotropic glutamate receptors are involved in the proteolysis of APP and sAPP release.
32. Conn, P. J. & Pin, J. P. Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* **37**, 205–237 (1997).
33. Schoepp, D. D., Jane, D. E. & Monn, J. A. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology* **38**, 1431–1476 (1999).
34. Pinheiro, P. S. & Mulle, C. Presynaptic glutamate receptors: physiological functions and mechanisms of action. *Nature Rev. Neurosci.* **9**, 423–436 (2008).
35. Schoepp, D. D. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. *J. Pharmacol. Exp. Ther.* **299**, 12–20 (2001).
36. Ferraguti, F., Baldani-Guerra, B., Corsi, M., Nakanishi, S. & Corti, C. Activation of the extracellular signal-regulated kinase 2 by metabotropic glutamate receptors. *Eur. J. Neurosci.* **11**, 2073–2082 (1999).
37. Phillips, T., Barnes, A., Scott, S., Emson, P. & Rees, S. Human metabotropic glutamate receptor 2 couples to the MAP kinase cascade in chinese hamster ovary cells. *Neuroreport* **9**, 2335–2339 (1998).
38. Albasanz, J. L., Dalfo, E., Ferrer, I. & Martin, M. Impaired metabotropic glutamate receptor/phospholipase C signaling pathway in the cerebral cortex in Alzheimer's disease and dementia with Lewy bodies correlates with stage of Alzheimer's disease-related changes. *Neurobiol. Dis.* **20**, 685–693 (2005).
39. Westmark, C. J., Westmark, P. R. & Malter, J. S. MPEP reduces seizure severity in Fmr-1 KO mice over expressing human Abeta. *Int. J. Clin. Exp. Pathol.* **3**, 56–68 (2009).
40. Lee, H. G. *et al.* Aberrant expression of metabotropic glutamate receptor 2 in the vulnerable neurons of Alzheimer's disease. *Acta Neuropathol.* **107**, 365–371 (2004).
41. Lee, H. G. *et al.* The effect of mGluR2 activation on signal transduction pathways and neuronal cell survival. *Brain Res.* **1249**, 244–250 (2009).
42. Kim, S. H. *et al.* Group II metabotropic glutamate receptor stimulation triggers production and release of Alzheimer's amyloid β₄₂ from isolated intact nerve terminals. *J. Neurosci.* **30**, 3870–3875 (2010).
43. Kim, J. *et al.* Abeta40 inhibits amyloid deposition *in vivo*. *J. Neurosci.* **27**, 627–633 (2007).
44. Caughey, B. & Lansbury, P. T. Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annu. Rev. Neurosci.* **26**, 267–298 (2003).
45. Younkin, S. G. The role of A beta 42 in Alzheimer's disease. *J. Physiol. Paris* **92**, 289–292 (1998).
46. Fryer, J. D. & Holtzman, D. M. The bad seed in Alzheimer's disease. *Neuron* **47**, 167–168 (2005).
47. Higgins, G. A. *et al.* Pharmacological manipulation of mGlu2 receptors influences cognitive performance in the rodent. *Neuropharmacology* **46**, 907–917 (2004).
48. Blin, J. *et al.* Loss of brain 5-HT₂ receptors in Alzheimer's disease. *In vivo* assessment with positron emission tomography and [18F]setoperone. *Brain* **116**, 497–510 (1993).
49. Holmes, C., Arranz, M. J., Powell, J. F., Collier, D. A. & Lovestone, S. 5-HT_{2A} and 5-HT_{2C} receptor polymorphisms and psychopathology in late onset Alzheimer's disease. *Hum. Mol. Genet.* **7**, 1507–1509 (1998).
50. Assal, F. *et al.* Association of the serotonin transporter and receptor gene polymorphisms in neuropsychiatric symptoms in Alzheimer disease. *Arch. Neurol.* **61**, 1249–1253 (2004).
51. Holmes, C., Arranz, M., Collier, D., Powell, J. & Lovestone, S. Depression in Alzheimer's disease: the effect of serotonin receptor gene variation. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **119B**, 40–43 (2003).
52. Nitsch, R. M., Deng, M., Growdon, J. H. & Wurtman, R. J. Serotonin 5-HT_{2A} and 5-HT_{2C} receptors stimulate amyloid precursor protein ectodomain secretion. *J. Biol. Chem.* **271**, 4188–4194 (1996).

53. Arjona, A. A., Pooler, A. M., Lee, R. K. & Wurtman, R. J. Effect of a 5-HT_{2C} serotonin agonist, dexnorfenfluramine, on amyloid precursor protein metabolism in guinea pigs. *Brain Res.* **951**, 135–140 (2002).
54. Medhurst, A. D., Lezoualc'h, F., Fischmeister, R., Middlemiss, D. N. & Sanger, G. J. Quantitative mRNA analysis of five C-terminal splice variants of the human 5-HT₄ receptor in the central nervous system by TaqMan real time RT-PCR. *Brain Res. Mol. Brain Res.* **90**, 125–134 (2001).
55. Cachard-Chastel, M. *et al.* 5-HT₄ receptor agonists increase sAPP α levels in the cortex and hippocampus of male C57BL/6j mice. *Br. J. Pharmacol.* **150**, 883–892 (2007).
56. Consolo, S., Annibaldi, S., Giorgi, S., Russi, G. & Ladinsky, H. 5-HT₄ receptor stimulation facilitates acetylcholine release in rat frontal cortex. *Neuroreport* **5**, 1230–1232 (1994).
57. Cho, S. & Hu, Y. Activation of 5-HT₄ receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. *Exp. Neurol.* **203**, 274–278 (2007).
58. Reynolds, G. P. *et al.* 5-Hydroxytryptamine (5-HT)₄ receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases. *Br. J. Pharmacol.* **114**, 993–998 (1995).
59. Ruat, M. *et al.* A novel rat serotonin (5-HT₆) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem. Biophys. Res. Commun.* **193**, 268–276 (1993).
60. Kohen, R. *et al.* Cloning, characterization, and chromosomal localization of a human 5-HT₆ serotonin receptor. *J. Neurochem.* **66**, 47–56 (1996).
61. Foley, A. G. *et al.* The 5-HT₆ receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. *Neuropsychopharmacology* **29**, 93–100 (2004).
62. Mitchell, E. S. & Neumaier, J. F. 5-HT₆ receptors: a novel target for cognitive enhancement. *Pharmacol. Ther.* **108**, 320–333 (2005).
63. Geldenhuys, W. J. & Van der Schyf, C. J. The serotonin 5-HT₆ receptor: a viable drug target for treating cognitive deficits in Alzheimer's disease. *Expert Rev. Neurother.* **9**, 1075–1085 (2009).
64. Upton, N., Chuang, T. T., Hunter, A. J. & Virley, D. J. 5-HT₆ receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. *Neurotherapeutics* **5**, 458–469 (2008).
65. Tsai, S. J., Liu, H. C., Liu, T. Y., Wang, Y. C. & Hong, C. J. Association analysis of the 5-HT₆ receptor polymorphism C267T in Alzheimer's disease. *Neurosci. Lett.* **276**, 138–139 (1999).
66. Lorke, D. E., Lu, G., Cho, E. & Yew, D. T. Serotonin 5-HT_{2A} and 5-HT₆ receptors in the prefrontal cortex of Alzheimer and normal aging patients. *BMC Neurosci.* **7**, 36 (2006).
67. Lezoualc'h, F., Engert, S., Berning, B. & Behl, C. Corticotropin-releasing hormone-mediated neuroprotection against oxidative stress is associated with the increased release of non-amyloidogenic amyloid beta precursor protein and with the suppression of nuclear factor-kappaB. *Mol. Endocrinol.* **14**, 147–159 (2000).
68. Bissette, G., Reynolds, G. P., Kilts, C. D., Widerlov, E. & Nemeroff, C. B. Corticotropin-releasing factor-like immunoreactivity in senile dementia of the Alzheimer type. Reduced cortical and striatal concentrations. *JAMA* **254**, 3067–3069 (1985).
69. De Souza, E. B., Whitehouse, P. J., Kuhar, M. J., Price, D. L. & Vale, W. W. Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease. *Nature* **319**, 593–595 (1986).
70. Pomara, N. *et al.* CSF corticotropin-releasing factor (CRF) in Alzheimer's disease: its relationship to severity of dementia and monoamine metabolites. *Biol. Psychiatry* **26**, 500–504 (1989).
71. Behan, D. P. *et al.* Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. *Nature* **378**, 284–287 (1995).
72. Joo, K. M. *et al.* Distribution of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors (VPAC1, VPAC2, and PAC1 receptor) in the rat brain. *J. Comp. Neurol.* **476**, 388–413 (2004).
73. Sacchetti, B. *et al.* Pituitary adenylate cyclase-activating polypeptide hormone (PACAP) at very low dosages improves memory in the rat. *Neurobiol. Learn. Mem.* **76**, 1–6 (2001).
74. Kojro, E. *et al.* The neuropeptide PACAP promotes the alpha-secretase pathway for processing the Alzheimer amyloid precursor protein. *FASEB J.* **20**, 512–514 (2006).
75. Dogrukol-Ak, D., Tore, F. & Tuncel, N. Passage of VIP/PACAP/secretin family across the blood-brain barrier: therapeutic effects. *Curr. Pharm. Des.* **10**, 1325–1340 (2004).
76. Vassar, R., Kovacs, D. M., Yan, R. & Wong, P. C. The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. *J. Neurosci.* **29**, 12787–12794 (2009).
77. De Strooper, B., Vassar, R. & Golde, T. The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nature Rev. Neurol.* **6**, 99–107 (2010).
78. Teng, L., Zhao, J., Wang, F., Ma, L. & Pei, G. A GPCR/secretase complex regulates beta- and gamma-secretase specificity for Abeta production and contributes to AD pathogenesis. *Cell Res.* **20**, 138–153 (2010).
79. Mathieu-Kia, A. M., Fan, L. Q., Kreek, M. J., Simon, E. J. & Hiller, J. M. Mu-, delta- and kappa-opioid receptor populations are differentially altered in distinct areas of postmortem brains of Alzheimer's disease patients. *Brain Res.* **893**, 121–134 (2001).
80. Ni, Y. *et al.* Activation of beta2-adrenergic receptor stimulates gamma-secretase activity and accelerates amyloid plaque formation. *Nature Med.* **12**, 1390–1396 (2006).
- This study demonstrates that the β_2 -AR regulates the localization of the γ -secretase complex, thereby regulating the amyloidogenic processing of APP and exacerbating the amyloid pathology in an AD mouse model.**
81. Meilandt, W. J. *et al.* Enkephalin elevations contribute to neuronal and behavioral impairments in a transgenic mouse model of Alzheimer's disease. *J. Neurosci.* **28**, 5007–5017 (2008).
82. Diez, M. *et al.* Neuropeptide alterations in the hippocampal formation and cortex of transgenic mice overexpressing beta-amyloid precursor protein (APP) with the Swedish double mutation (APP23). *Neurobiol. Dis.* **14**, 579–594 (2003).
83. Williams, J. T., Christie, M. J. & Manzoni, O. Cellular and synaptic adaptations mediating opioid dependence. *Physiol. Rev.* **81**, 299–343 (2001).
84. von Zastrow, M., Svings, A., Haberkost-Debic, H. & Evans, C. Regulated endocytosis of opioid receptors: cellular mechanisms and proposed roles in physiological adaptation to opiate drugs. *Curr. Opin. Neurobiol.* **13**, 348–353 (2003).
85. Jansen, K. L., Faull, R. L., Dragunow, M. & Synek, B. L. Alzheimer's disease: changes in hippocampal N-methyl-D-aspartate, quisqualate, neurotensin, adenosine, benzodiazepine, serotonin and opioid receptors — an autoradiographic study. *Neuroscience* **39**, 613–627 (1990).
86. De Strooper, B., Aph-1, Pen-2, and Nicastrin with Presenilin generate an active gamma-Secretase complex. *Neuron* **38**, 9–12 (2003).
87. Langui, D. *et al.* Subcellular topography of neuronal Abeta peptide in APPxPS1 transgenic mice. *Am. J. Pathol.* **165**, 1465–1477 (2004).
88. Pasternak, S. H. *et al.* Presenilin-1, nicastrin, amyloid precursor protein, and gamma-secretase activity are co-localized in the lysosomal membrane. *J. Biol. Chem.* **278**, 26687–26694 (2003).
89. Kalaria, R. N. *et al.* Adrenergic receptors in aging and Alzheimer's disease: increased beta 2-receptors in prefrontal cortex and hippocampus. *J. Neurochem.* **53**, 1772–1781 (1989).
90. Yu, J. T. *et al.* Polymorphisms at the beta2-adrenergic receptor gene influence Alzheimer's disease susceptibility. *Brain Res.* **1210**, 216–222 (2008).
91. Uhlenbrock, K., Gassenhuber, H. & Kostenis, E. Sphingosine 1-phosphate is a ligand of the human gpr6, gpr6 and gpr12 family of constitutively active G protein-coupled receptors. *Cell Signal* **14**, 941–953 (2002).
92. Thathiah, A. *et al.* The orphan G protein-coupled receptor 3 modulates amyloid-beta peptide generation in neurons. *Science* **323**, 946–951 (2009).
- This study demonstrates that the orphan GPCR GPR3 regulates the *in vitro* and *in vivo* amyloidogenic proteolysis of APP through modulation of the localization and/or activity of the γ -secretase complex in the absence of an effect on Notch processing.**
93. Valverde, O. *et al.* GPR3 receptor, a novel actor in the emotional-like responses. *PLoS One* **4**, e4704 (2009).
94. Iismaa, T. P. *et al.* Isolation and chromosomal localization of a novel human G-protein-coupled receptor (GPR3) expressed predominantly in the central nervous system. *Genomics* **24**, 391–394 (1994).
95. Tanaka, S., Ishii, K., Kasai, K., Yoon, S. O. & Saeki, Y. Neural Expression of G. Protein-coupled Receptors GPR3, GPR6, and GPR12 Up-regulates Cyclic AMP Levels and Promotes Neurite Outgrowth. *J. Biol. Chem.* **282**, 10506–10515 (2007).
96. Radde, R. *et al.* Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep.* **7**, 940–946 (2006).
97. Horuk, R. *et al.* Expression of chemokine receptors by subsets of neurons in the central nervous system. *J. Immunol.* **158**, 2882–2890 (1997).
98. Xia, M., Qin, S., McNamara, M., Mackay, C. & Hyman, B. T. Interleukin-8 receptor B immunoreactivity in brain and neuritic plaques of Alzheimer's disease. *Am. J. Pathol.* **150**, 1267–1274 (1997).
99. Bakshi, P., Margenthaler, E., Laporte, V., Crawford, F. & Mullan, M. Novel role of CXCR2 in regulation of gamma-secretase activity. *ACS Chem. Biol.* **3**, 777–789 (2008).
100. Bakshi, P., Margenthaler, E., Reed, J., Crawford, F. & Mullan, M. Depletion of CXCR2 inhibits gamma-secretase activity and amyloid-beta production in a murine model of Alzheimer's disease. *Cytokine* **15** Nov 2010 (doi:10.1016/j.cyt.2010.10.008) [epub ahead of print].
101. Liao, Y. F., Wang, B. J., Cheng, H. T., Kuo, L. H. & Wolfe, M. S. Tumor necrosis factor-alpha, interleukin-1beta, and interferon-gamma stimulate gamma-secretase-mediated cleavage of amyloid precursor protein through a JNK-dependent MAPK pathway. *J. Biol. Chem.* **279**, 49523–49532 (2004).
102. Kuo, L. H. *et al.* Tumor necrosis factor-alpha-elicited stimulation of gamma-secretase is mediated by c-Jun N-terminal kinase-dependent phosphorylation of presenilin and nicastrin. *Mol. Biol. Cell* **19**, 4201–4212 (2008).
103. Lambert, M. P. *et al.* Diffusible, nonfibrillar ligands derived from Abeta1–42 are potent central nervous system neurotoxins. *Proc. Natl Acad. Sci. USA* **95**, 6448–6453 (1998).
104. Shenoy, U. V., Richards, E. M., Huang, X. C. & Summers, C. Angiotensin II type 2 receptor-mediated apoptosis of cultured neurons from newborn rat brain. *Endocrinology* **140**, 500–509 (1999).
105. Ichiki, T. *et al.* Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. *Nature* **377**, 748–750 (1995).
106. Vervoort, V. S. *et al.* AGTR2 mutations in X-linked mental retardation. *Science* **296**, 2401–2403 (2002).
107. AbdAlia, S., Lother, H., Abdel-tawab, A. M. & Quittner, U. The angiotensin II AT2 receptor is an AT1 receptor antagonist. *J. Biol. Chem.* **276**, 39721–39726 (2001).
108. Ferrari-DiLeo, G. & Flynn, D. D. Diminished muscarinic receptor-stimulated [3H]-PIP2 hydrolysis in Alzheimer's disease. *Life Sci.* **53**, PL439–444 (1993).
109. Tsang, S. W. *et al.* Impaired coupling of muscarinic M1 receptors to G-proteins in the neocortex is associated with severity of dementia in Alzheimer's disease. *Neurobiol. Aging* **27**, 1216–1223 (2006).
110. AbdAlia, S. *et al.* Angiotensin II AT2 receptor oligomers mediate G-protein dysfunction in an animal model of Alzheimer disease. *J. Biol. Chem.* **284**, 6554–6565 (2009).
111. Thathiah, A. & De Strooper, B. G protein-coupled receptors, cholinergic dysfunction, and Abeta toxicity in Alzheimer's disease. *Sci. Signal* **2**, re8 (2009).
112. Fredholm, B. B., AP, I. J., Jacobson, K. A., Klotz, K. N. & Linden, J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* **53**, 527–552 (2001).
113. Angulo, E. *et al.* A1 adenosine receptors accumulate in neurodegenerative structures in Alzheimer disease and mediate both amyloid precursor protein processing and tau phosphorylation and translocation. *Brain Pathol.* **13**, 440–451 (2003).

114. Albasanz, J. L., Perez, S., Barrachina, M., Ferrer, I. & Martin, M. Up-regulation of adenosine receptors in the frontal cortex in Alzheimer's disease. *Brain Pathol.* **18**, 211–219 (2008).
115. Wang, J. H., Ma, Y. Y. & van den Buuse, M. Improved spatial recognition memory in mice lacking adenosine A2A receptors. *Exp. Neurol.* **199**, 438–445 (2006).
116. Gimenez-Llort, L. *et al.* Working memory deficits in transgenic rats overexpressing human adenosine A2A receptors in the brain. *Neurobiol. Learn. Mem.* **87**, 42–56 (2007).
117. Dall'Igna, O. P., Porciuncula, L. O., Souza, D. O., Cunha, R. A. & Lara, D. R. Neuroprotection by caffeine and adenosine A2A receptor blockade of beta-amyloid neurotoxicity. *Br. J. Pharmacol.* **138**, 1207–1209 (2003).
118. Dall'Igna, O. P. *et al.* Caffeine and adenosine A(2a) receptor antagonists prevent beta-amyloid (25–35)-induced cognitive deficits in mice. *Exp. Neurol.* **203**, 241–245 (2007).
119. Arendash, G. W. *et al.* Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production. *Neuroscience* **142**, 941–952 (2006).
120. Canas, P. M. *et al.* Adenosine A2A receptor blockade prevents synaptotoxicity and memory dysfunction caused by beta-amyloid peptides via p38 mitogen-activated protein kinase pathway. *J. Neurosci.* **29**, 14741–14751 (2009).
121. Querfurth, H. W., Jiang, J., Geiger, J. D. & Selkoe, D. J. Caffeine stimulates amyloid beta-peptide release from beta-amyloid precursor protein-transfected HEK293 cells. *J. Neurochem.* **69**, 1580–1591 (1997).
122. Broad, R. M. & Fredholm, B. B. A1, but not A2A, adenosine receptors modulate electrically stimulated [14C]acetylcholine release from rat cortex. *J. Pharmacol. Exp. Ther.* **277**, 193–197 (1996).
123. Carter, A. J., O'Connor, W. T., Carter, M. J. & Ungerstedt, U. Caffeine enhances acetylcholine release in the hippocampus *in vivo* by a selective interaction with adenosine A1 receptors. *J. Pharmacol. Exp. Ther.* **273**, 637–642 (1995).
124. Frautschy, S. A. *et al.* Microglial response to amyloid plaques in APPsw transgenic mice. *Am. J. Pathol.* **152**, 307–317 (1998).
125. Babcock, A. A., Kuziel, W. A., Rivest, S. & Owens, T. Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J. Neurosci.* **23**, 7922–7930 (2003).
126. Ishizuka, K. *et al.* Identification of monocyte chemoattractant protein-1 in senile plaques and reactive microglia of Alzheimer's disease. *Psychiatry Clin. Neurosci.* **51**, 135–138 (1997).
127. Smits, H. A. *et al.* Amyloid-beta-induced chemokine production in primary human macrophages and astrocytes. *J. Neuroimmunol.* **127**, 160–168 (2002).
128. El Khoury, J. *et al.* Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. *Nature Med.* **13**, 432–438 (2007).
129. Chapman, G. A. *et al.* Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage. *J. Neurosci.* **20**, RC87 (2000).
130. Harrison, J. K. *et al.* Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. *Proc. Natl Acad. Sci. USA* **95**, 10896–10901 (1998).
131. Fuhrmann, M. *et al.* Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. *Nature Neurosci.* **13**, 411–413 (2010).
132. Grathwohl, S. A. *et al.* Formation and maintenance of Alzheimer's disease beta-amyloid plaques in the absence of microglia. *Nature Neurosci.* **12**, 1361–1363 (2009).
133. Epelbaum, J., Dournaud, P., Fodor, M. & Viollet, C. The neurobiology of somatostatin. *Crit. Rev. Neurobiol.* **8**, 25–44 (1994).
134. Patel, Y. C. Somatostatin and its receptor family. *Front. Neuroendocrinol.* **20**, 157–198 (1999).
135. Kumar, U. Expression of somatostatin receptor subtypes (SSTR1–5) in Alzheimer's disease brain: an immunohistochemical analysis. *Neuroscience* **134**, 525–538 (2005).
136. Tamminga, C. A., Foster, N. L., Fedio, P., Bird, E. D. & Chase, T. N. Alzheimer's disease: low cerebral somatostatin levels correlate with impaired cognitive function and cortical metabolism. *Neurology* **37**, 161–165 (1987).
137. Dournaud, P., Delaere, P., Hauw, J. J. & Epelbaum, J. Differential correlation between neurochemical deficits, neuropathology, and cognitive status in Alzheimer's disease. *Neurobiol. Aging* **16**, 817–823 (1995).
138. Davies, P., Katzman, R. & Terry, R. D. Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementia. *Nature* **288**, 279–280 (1980).
139. Saito, T. *et al.* Somatostatin regulates brain amyloid beta peptide Abeta42 through modulation of proteolytic degradation. *Nature Med.* **11**, 434–439 (2005).
- This study demonstrates that somatostatin, a neuropeptide that binds to the SSTRs in the brain, regulates the activity of neprilysin, one of the major amyloid-β-degrading enzymes.**
140. Iwata, N. *et al.* Metabolic regulation of brain Abeta by neprilysin. *Science* **292**, 1550–1552 (2001).
141. Ramos, B. *et al.* Early neuropathology of somatostatin/NPY GABAergic cells in the hippocampus of a PS1xAPP transgenic model of Alzheimer's disease. *Neurobiol. Aging* **27**, 1658–1672 (2006).
142. Horgan, J., Miguel-Hidalgo, J. J., Thrasher, M. & Bissette, G. Longitudinal brain corticotropin releasing factor and somatostatin in a transgenic mouse (TG2576) model of Alzheimer's disease. *J. Alzheimers Dis.* **12**, 115–127 (2007).
143. Slack, B. E. & Wurtman, R. J. In *Research Progress in Alzheimer's Disease and Dementia* (ed. Sun, M.-K.) 1–25 (Nova Science Publishers, New York, 2006).
- This is an excellent review that examines the effects of neurotransmitters, growth factors and cytokines on the synthesis and metabolism of APP.**
144. Lemere, C. A. & Masliah, E. Can Alzheimer disease be prevented by amyloid-beta immunotherapy? *Nature Rev. Neurol.* **6**, 108–119 (2010).
145. Selkoe, D. J. The molecular pathology of Alzheimer's disease. *Neuron* **6**, 487–498 (1991).
146. Hardy, J. A. & Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**, 184–185 (1992).
147. Bartus, R. T., Dean, R. L. 3rd, Beer, B. & Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408–414 (1982).
148. Davies, P. & Maloney, A. J. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**, 1403 (1976).
149. Whitehouse, P. J. *et al.* Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**, 1237–1239 (1982).
150. Wolf, N. J. The critical role of cholinergic basal forebrain neurons in morphological change and memory encoding: a hypothesis. *Neurobiol. Learn. Mem.* **66**, 258–266 (1996).
151. Tzavara, E. T. *et al.* Dysregulated hippocampal acetylcholine neurotransmission and impaired cognition in M2, M4 and M2/M4 muscarinic receptor knockout mice. *Mol. Psychiatry* **8**, 673–679 (2003).
152. Zhang, W. *et al.* Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. *J. Neurosci.* **22**, 1709–1717 (2002).
153. Sadot, E. *et al.* Activation of m1 muscarinic acetylcholine receptor regulates tau phosphorylation in transfected PC12 cells. *J. Neurochem.* **66**, 877–880 (1996).
154. Oddo, S. *et al.* Chronic nicotine administration exacerbates tau pathology in a transgenic model of Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **102**, 3046–3051 (2005).
155. Haan, M. N. Therapy Insight: type 2 diabetes mellitus and the risk of late-onset Alzheimer's disease. *Nature Clin. Pract. Neurol.* **2**, 159–166 (2006).
156. Biessels, G. J. *et al.* Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. *Diabetes* **45**, 1259–1266 (1996).
157. Zhao, W. *et al.* Brain insulin receptors and spatial memory. Correlated changes in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats. *J. Biol. Chem.* **274**, 34893–34902 (1999).
158. Zhao, W. Q., Chen, H., Quon, M. J. & Alkon, D. L. Insulin and the insulin receptor in experimental models of learning and memory. *Eur. J. Pharmacol.* **490**, 71–81 (2004).
159. Carro, E. & Torres-Aleman, I. The role of insulin and insulin-like growth factor 1 in the molecular and cellular mechanisms underlying the pathology of Alzheimer's disease. *Eur. J. Pharmacol.* **490**, 127–133 (2004).
160. Beeri, M. S. *et al.* Insulin in combination with other diabetes medication is associated with less Alzheimer neuropathology. *Neurology* **71**, 750–757 (2008).
161. Plastino, M. *et al.* Effects of insulin therapy on cognitive impairment in patients with Alzheimer disease and diabetes mellitus type-2. *J. Neurol. Sci.* **288**, 112–116 (2010).
162. Li, L. & Holscher, C. Common pathological processes in Alzheimer disease and type 2 diabetes: a review. *Brain Res. Rev.* **56**, 384–402 (2007).
163. Abbas, T., Faivre, E. & Holscher, C. Impairment of synaptic plasticity and memory formation in GLP-1 receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease. *Behav. Brain Res.* **205**, 265–271 (2009).
164. Perry, T. *et al.* Glucagon-like peptide-1 decreases endogenous amyloid-beta peptide (Abeta) levels and protects hippocampal neurons from death induced by Abeta and iron. *J. Neurosci. Res.* **72**, 603–612 (2003).
165. Gault, V. A. & Holscher, C. GLP-1 agonists facilitate hippocampal LTP and reverse the impairment of LTP induced by beta-amyloid. *Eur. J. Pharmacol.* **587**, 112–117 (2008).
166. Wang, X. H. *et al.* Val8-glucagon-like peptide-1 protects against Abeta1-40-induced impairment of hippocampal late-phase long-term potentiation and spatial learning in rats. *Neuroscience* **170**, 1239–1248 (2010).
167. Gao, H. *et al.* GLP-1 amplifies insulin signaling by up-regulation of IRbeta, IRS-1 and Glut4 in 3T3-L1 adipocytes. *Endocrine* **32**, 90–95 (2007).
168. Wheeler, M. B. *et al.* Functional expression of the rat glucagon-like peptide-I receptor, evidence for coupling to both adenyl cyclase and phospholipase-C. *Endocrinology* **133**, 57–62 (1993).
169. Montrose-Rafizadeh, C. *et al.* Pancreatic glucagon-like peptide-1 receptor couples to multiple G proteins and activates mitogen-activated protein kinase pathways in Chinese hamster ovary cells. *Endocrinology* **140**, 1132–1140 (1999).
170. Buteau, J., Roduit, R., Susini, S. & Prentki, M. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. *Diabetologia* **42**, 856–864 (1999).
171. Holz, G. G., Leech, C. A. & Habener, J. F. Activation of a cAMP-regulated Ca²⁺-signaling pathway in pancreatic beta-cells by the insulinotropic hormone glucagon-like peptide-1. *J. Biol. Chem.* **270**, 17749–17757 (1995).
172. Doring, M. J. *et al.* Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nature Med.* **9**, 1173–1179 (2003).
173. Cooper, G. J. *et al.* Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc. Natl Acad. Sci. USA* **84**, 8628–8632 (1987).
174. Dore, S., Kar, S. & Quirion, R. Insulin-like growth factor 1 protects and rescues hippocampal neurons against beta-amyloid- and human amylin-induced toxicity. *Proc. Natl Acad. Sci. USA* **94**, 4772–4777 (1997).
175. Jhamandas, J. H. & MacTavish, D. Antagonist of the amylin receptor blocks beta-amyloid toxicity in rat cholinergic basal forebrain neurons. *J. Neurosci.* **24**, 5579–5584 (2004).
176. Takeda, S. *et al.* Diabetes-accelerated memory dysfunction via cerebrovascular inflammation and Abeta deposition in an Alzheimer mouse model with diabetes. *Proc. Natl Acad. Sci. USA* **107**, 7036–7041 (2010).
177. Nitsch, R. M., Deng, M., Tennis, M., Schoenfeld, D. & Growdon, J. H. The selective muscarinic M1 agonist AF102B decreases levels of total Abeta in cerebrospinal fluid of patients with Alzheimer's disease. *Ann. Neurol.* **48**, 913–918 (2000).
178. Beach, T. G., Walker, D. G., Potter, P. E., Sue, L. I. & Fisher, A. Reduction of cerebrospinal fluid amyloid beta after systemic administration of M1 muscarinic agonists. *Brain Res.* **905**, 220–223 (2001).

179. Kirazov, L., Loffler, T., Schliebs, R. & Bigl, V. Glutamate-stimulated secretion of amyloid precursor protein from cortical rat brain slices. *Neurochem. Int.* **30**, 557–563 (1997).
180. Nitsch, R. M., Deng, A., Wurtman, R. J. & Growdon, J. H. Metabotropic glutamate receptor subtype mGluR1alpha stimulates the secretion of the amyloid beta-protein precursor ectodomain. *J. Neurochem.* **69**, 704–712 (1997).
181. Maillet, M. *et al.* Crosstalk between Rap1 and Rac regulates secretion of sAPPalpha. *Nature Cell Biol.* **5**, 633–639 (2003).
182. Robert, S. J. & Lezoualc'h, F. Distinct functional effects of human 5-HT4 receptor isoforms on beta-amyloid secretion. *Neurodegener. Dis.* **5**, 163–165 (2008).
183. Xia, M. & Hyman, B. T. GROalpha/KC, a chemokine receptor CXCR2 ligand, can be a potent trigger for neuronal ERK1/2 and PI-3 kinase pathways and for tau hyperphosphorylation—a role in Alzheimer's disease? *J. Neuroimmunol.* **122**, 55–64 (2002).
184. AbdAlla, S. *et al.* Dominant negative AT2 receptor oligomers induce G-protein arrest and symptoms of neurodegeneration. *J. Biol. Chem.* **284**, 6566–6574 (2009).
185. Cunha, G. M. *et al.* Adenosine A2A receptor blockade prevents memory dysfunction caused by beta-amyloid peptides but not by scopolamine or MK-801. *Exp. Neurol.* **210**, 776–781 (2008).
186. Nordberg, A. *et al.* Chronic nicotine treatment reduces beta-amyloidosis in the brain of a mouse model of Alzheimer's disease (APPsw). *J. Neurochem.* **81**, 655–658 (2002).
187. Hellstrom-Lindahl, E. *et al.* Nicotine reduces A beta in the brain and cerebral vessels of APPsw mice. *Eur. J. Neurosci.* **19**, 2703–2710 (2004).
188. Wang, H. Y., Li, W., Benedetti, N. J. & Lee, D. H. Alpha 7 nicotinic acetylcholine receptors mediate beta-amyloid peptide-induced tau protein phosphorylation. *J. Biol. Chem.* **278**, 31547–31553 (2003).
189. Dineley, K. T. *et al.* Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal alpha7 nicotinic acetylcholine receptors: *in vitro* and *in vivo* mechanisms related to Alzheimer's disease. *J. Neurosci.* **21**, 4125–4133 (2001).
190. Unger, C., Svedberg, M. M., Yu, W. F., Hedberg, M. M. & Nordberg, A. Effect of subchronic treatment of memantine, galantamine, and nicotine in the brain of Tg2576 (APPsw) transgenic mice. *J. Pharmacol. Exp. Ther.* **317**, 30–36 (2006).
191. Pettit, D. L., Shao, Z. & Yakel, J. L. beta-Amyloid(1–42) peptide directly modulates nicotinic receptors in the rat hippocampal slice. *J. Neurosci.* **21**, RC120 (2001).
192. Dziejczapolski, G., Glogowski, C. M., Maslah, E. & Heinemann, S. F. Deletion of the alpha 7 nicotinic acetylcholine receptor gene improves cognitive deficits and synaptic pathology in a mouse model of Alzheimer's disease. *J. Neurosci.* **29**, 8805–8815 (2009).
193. Ray, B., Banerjee, P. K., Greig, N. H. & Lahiri, D. K. Memantine treatment decreases levels of secreted Alzheimer's amyloid precursor protein (APP) and amyloid beta (A beta) peptide in the human neuroblastoma cells. *Neurosci. Lett.* **470**, 1–5 (2010).
194. Alley, G. M. *et al.* Memantine lowers amyloid-beta peptide levels in neuronal cultures and in APP/PS1 transgenic mice. *J. Neurosci. Res.* **88**, 143–154 (2010).
195. Minkeviciene, R., Banerjee, P. & Tanila, H. Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease. *J. Pharmacol. Exp. Ther.* **311**, 677–682 (2004).
196. Scholtzova, H. *et al.* Memantine leads to behavioral improvement and amyloid reduction in Alzheimer's disease-model transgenic mice shown as by micromagnetic resonance imaging. *J. Neurosci. Res.* **86**, 2784–2791 (2008).
197. Brandenburg, L. O. *et al.* Involvement of formyl-peptide-receptor-like-1 and phospholipase D in the internalization and signal transduction of amyloid beta 1–42 in glial cells. *Neuroscience* **156**, 266–276 (2008).

Acknowledgements

Work in the laboratory was supported by the Flanders Institute for Biotechnology (VIB), Fonds voor Wetenschappelijk onderzoek (FWO) and Stichting Alzheimer Onderzoek-Fondation pour la recherche de la maladie d'Alzheimer (SAO-FRMA) (grant cycle 2008–2009), the Federal Office for Scientific Affairs, Belgium (IUAP P6/43), a Methusalem grant of the Catholic University of Leuven (KUL) and the Flemish Government, and Memosad (FZ-2007-200611) of the European Union. B.D.S. is supported by an Arthur Bax and Anna Vanluffelen Chair for Alzheimer's Disease.

Competing interests statement

The authors declare no competing financial interests.