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Galanin in Alzheimer's disease: Neuroinhibitory or neuroprotective?

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Abstract

Galanin (GAL) and GAL receptors (GALRs) are overexpressed in degenerating brain regions associated with cognitive decline in Alzheimer's disease (AD). The functional consequences of GAL plasticity in AD are unclear. GAL inhibits cholinergic transmission in the hippocampus and impairs spatial memory in rodent models, suggesting GAL overexpression exacerbates cognitive impairment in AD. By contrast, gene expression profiling of individual cholinergic basal forebrain (CBF) neurons aspirated from AD tissue revealed that GAL hyperinnervation positively regulates mRNAs that promote CBF neuronal function and survival. GAL also exerts neuroprotective effects in rodent models of neurotoxicity. These data support the growing concept that GAL overexpression preserves CBF neuron function which in turn may slow the onset of AD symptoms. Further elucidation of GAL activity in selectively vulnerable brain regions will help gauge the therapeutic potential of GALR ligands for the treatment of AD. (Part of a Multi-author Review)

Keywords

Galanin; Alzheimer's disease; cholinergic basal forebrain; hippocampus; plasticity

Introduction

The neuropeptide galanin (GAL) and its cognate G-protein-coupled receptors (GALR1–3) are widely distributed in the mammalian central nervous system (CNS) and modulate several ascending neurotransmitter systems including cholinergic, noradrenergic, serotonergic as well as neuroendocrine pathways [1–8]. Notably, GAL activity regulates cognitive behaviors mediated by the basal forebrain, amygdala, hippocampus, and entorhinal cortex [2,9–15]. GAL regulates cholinergic basal forebrain (CBF) neurons that provide the major cholinergic innervation to the cortex and hippocampus [16] and play a key role in memory and attentional functions [17–19]. CBF neurons undergo selective degeneration during the later stages of Alzheimer's disease (AD) that correlates with disease duration and degree of cognitive impairment [20]. Several groups have made the striking observation that hypertrophic GAL-containing fibers innervate surviving CBF neurons in the end-stage AD brain [21–23]. GAL levels are also increased throughout the cortex in AD [24,25], and GALR binding sites are amplified in the cortex, CBF, hippocampus, entorhinal cortex, and amygdala during the course of the disease [26–29]. However, the functional impact of GAL

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overexpression within the CBF in AD is unclear. For example, GAL inhibits acetylcholine (ACh) release in rodent hippocampal preparations, restricts long-term potentiation (LTP) and disrupts cognitive performance in animals [2,30,31], supporting the notion that CBF GAL fiber hypertrophy exacerbates the cholinergic deficit seen in AD. This hypothesis has been challenged by recent findings demonstrating that GAL protects the hippocampus from excitotoxic damage [32] and CBF septal neurons from amyloid toxicity [33]. These observations raise the possibility that GAL upregulation promotes cholinergic neuronal survival during the late stage of AD. In this regard, gene expression profiling of individual CBF neurons in AD tissue suggests that GAL hyperinnervation positively regulates mRNAs that promote cholinergic neuron function and survival [34,35]. This article reviews evidence supporting the concept that GAL overexpression plays a role in the survival of select neuronal populations associated with cognitive decline in AD.

Galanin in Alzheimer's disease

Galaninergic systems undergo hypertrophy in brain regions that mediate cognition and are prone to AD neuropathological damage. For instance, immunohistochemical studies of postmortem basal forebrain tissue from aged, cognitively intact subjects reveal a fine network of GAL-immunoreactive (-ir) fibers coursing through the CBF which often appear in close apposition to CBF perikarya or dendrites (Fig. 1A). In contrast, end-stage AD tissue displays a dense plexus of enlarged GAL-ir fibers hyperinnervating surviving CBF neocortical and hippocampal projection neurons located within the nucleus basalis (NB, Fig. 1B) and septal diagonal band complex, respectively [21–23]. GAL radioimmunoassay (RIA) studies of autopsied CBF tissue revealed a 2-fold increase in GAL peptide levels within the NB of late-stage AD subjects relative to controls [36], whereas quantitative *in vitro* autoradiographic imaging of [^{125}I]hGAL binding sites within the NB of pathologically defined early (mild) and late (severe) AD cases showed a significant increase in the density of GALR labeling within the anterior NB subfield of late AD subjects compared to controls and early AD subjects (Fig. 1C – E) [27]. In addition, a semi-quantitative immunohistochemical study of GAL-ir profiles in the NB of people clinically diagnosed with mild cognitive impairment, a putative preclinical AD stage [37] or mild AD revealed no evidence for GAL hyperinnervation of this CBF region during the prodromal or early stages of AD [38]. Taken together, these findings indicate that GAL fiber and receptor overexpression occurs within the anterior portion of the NB during the late stage of AD when CBF neuron degeneration is advanced.

There is evidence for GAL plasticity in other brain regions associated with cognitive dysfunction in AD. The noradrenergic locus coeruleus (LC), which similarly to the CBF contains long neocortical and hippocampal projection neurons that modulate memory and attention [39] and degenerate in AD [40], also exhibits prominent GAL-ir fiber hyperinnervation in postmortem AD tissue [41]. Two additional studies used GAL RIA to demonstrate a significant increase in GAL peptide concentration in frontal, temporal and parietal neocortical association areas in end-stage AD, but not in controls [24,25] or patients with schizophrenia [25]. Furthermore, *in vitro* autoradiographic studies revealed a significant increase in GALR occupancy in the deep layers of the frontal cortex of AD subjects [26]. GALR binding sites were also detected in all neocortical areas and in layer II of the entorhinal cortex, the uncus and the hippocampal-amygdala transition area in human brain tissue [28,29,42,43]. Autoradiographic localization of GALR binding sites within entorhinal cortex layer II is intriguing since these neurons provide the major glutamatergic excitatory input to the hippocampus (i.e., the perforant pathway) and degenerate very early in AD [44–46]. *In vitro* autoradiographic studies of [^{125}I]hGAL binding in control and AD subjects revealed an ~3-fold increase in GALR binding sites in entorhinal cortex layer II in early AD patients compared to those with late-stage AD or age-matched control subjects

[28]. [^{125}I]hGAL binding sites were also localized to the central nucleus and cortico-amygdaloid transition area of the amygdala, which have reciprocal connections with the basal forebrain, hippocampus and cortex. These regions play a pivotal role in higher-order cognitive processing and display extensive AD-related pathology early in the disease process [47–49]. Similar to findings in layer II of the entorhinal cortex, [^{125}I]hGAL binding was upregulated in the amygdala in early/probable AD but not during late-stage AD [28]. Hence, increased GALR binding occurs in select cognitive regions of the AD brain that are affected in the early stages of AD [45,46,49]. On the other hand, increased GALR binding and GAL fiber hyperinnervation are found within the CBF projection system only in the late stage of the disease when these neurons are succumbing to the disorder [21–23,27].

Potential triggers of galanin plasticity in AD

The pathophysiological factors that induce GAL plasticity in the AD brain have been a matter of great speculation. As discussed above, the spatiotemporal pattern of increased GAL binding suggests that GAL hypertrophy occurs in response to neuronal injury. Along these lines, GAL is dramatically upregulated following several experimental injury paradigms in the rat central and peripheral nervous systems, including olfactory bulbectomy [50], hypophysectomy [51], neurochemical dorsal raphe lesions [52], immunotoxic basal forebrain lesions [53], potassium chloride-mediated cortical spreading depression [54], global ischemia [55], and sciatic [56] or dorsal root sensory [57] nerve transaction. Collectively, these observations support the notion that GAL overexpression is triggered by neuronal damage, suggesting that GAL fiber hyperinnervation of cell groups such as the CBF and LC in AD represents an intrinsic cellular program aimed at neuron survival.

AD neurodegenerative lesions may also play a role in GAL fiber hypertrophy. For instance, human neuropathological studies have shown that AD-related neuritic plaques, which are composed chiefly of fibrillar deposits of β-amyloid (Aβ) [58], are also GAL-positive [59]. A role for Aβ deposition in triggering GAL upregulation is supported by studies using transgenic mouse models of AD, which display prominent amyloidosis. Older (26-month-old) mice that overexpress human amyloid precursor protein (APP) bearing the familial AD (FAD)-related V717F mutation exhibit amyloid plaque deposition in the hippocampal stratum lacunosum-moleculare subfield and entorhinal cortex concurrent with the appearance of dystrophic GAL-ir neurites in many of the plaques [60]. Occasional GAL-ir cell bodies were also observed in the hippocampus that were not evident in wild-type mice [60]. In addition, APP23 mice bearing two FAD-related APP mutations (V717I and K670N/M671L) exhibited an increase of dystrophic GAL fibers and GAL-ir neurites apposing amyloid plaques in the supragranular layer of the hippocampus and ventral neocortex of 27-month-old compared to 21-month-old mice [61]. Interestingly, GAL immunoreactivity was reduced in the dorsolateral neocortex of 27-month-old APP23 mice [61], suggesting a dynamic age-related reorganization of cortical GAL-containing projections in the face of mounting amyloid deposition. More recently, we examined Aβ and GAL immunoreactivity in the brains of transgenic mice carrying the human APP K670N/M671L (APPswe) and presenilin 1 PS1ΔE9 FAD mutations, which display accelerated plaque deposition compared to APP transgenic mice [62]. Co-labeling of cortical and hippocampal amyloid plaques with GAL revealed peptide containing dystrophic neurites as early as 3 months of age (Fig. 2A – C). Since neuron loss was not evident at this age [63,64], these observations suggest that GAL is triggered by amyloidosis-related neurotoxicity rather than frank neurodegeneration in transgenic animal models of AD. Whether amyloid plaque deposition, which is prominent in vulnerable cognitive brain regions in AD [65,66], also triggers GAL overexpression in these areas in the human condition remains an open question.

Neurofibrillary tangles (NFTs), filamentous deposits composed of aggregated tau microtubule-binding proteins [67,68], are also a cardinal neuropathological feature of AD. In the CBF, the evolution of NFT pathology within individual neurons follows a sequence of differentially expressed tau epitopes during the course of AD [69]. Using antibodies raised against different tau epitopes, we tested whether GAL plasticity is associated with the evolution of NFTs in CBF neurons in AD [70]. CBF neurons displaying the tau C3 epitope, a marker for early stage NFT formation, were often hyperinnervated by GAL-ir fibers (Fig. 2D), whereas CBF neurons displaying the tau epitope MN423, an end-stage NFT marker, were not associated with GAL (Fig. 2E). Single-cell gene expression studies have shown that the levels of mRNAs encoding select subclasses of protein phosphatase subunits (PP1α and PP1γ) are stable in GAL hyperinnervated but downregulated in non-hyper-innervated CBF neurons in AD[35].Reduced activity of PP1 and PP2A subunits is implicated in tau hyperphosphorylation, which precipitates NFT pathology and subsequent cytoskeletal destabilization in vulnerable neurons [71]. Taken together, these observations suggest that GAL remodeling may delay NFT pathology in CBF neurons in AD.

Neuronal origin of galanin hyperinnervation in AD

The source(s) of GAL hyperinnervation in AD remain unclear. For instance, it is unlikely that the few small GAL-ir neurons within the basal forebrain and preoptic area account for the rich galaninergic fiber plexus seen within this region of the human brain [72,73]. One potential source of GAL fiber innervation to the basal forebrain may be the LC[74–76].The coeruleo-forebrain pathway is well characterized in the mammalian CNS [77], and GAL-ir cells within the LC also exhibit enhanced GAL immunoreactivity in AD [41]. Although the human LC does not contain numerous GAL-ir cells [73] as compared to rodents [78,79], GAL-positive LC neurons are preserved in AD [75], suggesting a neuroprotective action for GAL as well as a source for GAL forebrain hyperinnervation. Another potential source of GAL fibers may emanate from the central nucleus of the amygdala and course through the basal forebrain en route to the substantia innominata, bed nucleus of the stria terminalis and hypothalamus [23]. While the precise cells of origin of this GAL-ir forebrain bundle remain to be determined, they may arise from the extended amygdaloid complex, which contains numerous GAL-ir cell bodies [23,75] and displays hypertrophy of GAL-ir fibers in AD [E. J. Mufson, unpublished observations].

Galanin plasticity as a detrimental factor in AD

The functional consequences of GAL plasticity in AD remain an area of intense interest. The majority of evidence concerning the effects of GAL overexpression in AD is derived from rodent studies showing that GAL inhibits ACh release in the hippocampus [1,2,80,81], restricts LTP [9,11,30], and disrupts cognitive performance on emotional and spatial memory tasks [15,31]. GAL inhibits the evoked release of ACh in the ventral hippocampus of the rat in a concentration-dependent manner and blocks the slow cholinergic excitatory post-synaptic potential (EPSP) induced by the release of endogenous ACh onto CA1 hippocampal pyramidal neurons [1]. Furthermore, microinjection of GAL into the medial septum/diagonal band complex or ventral hippocampus impairs cognitive performance on several spatial learning and working memory tasks in rats [15,31]. GAL interference of cholinergic transmission during these tasks is particularly evident in the presence of muscarinic ACh receptor antagonists or cholinergic immunotoxin lesions [31,81,82].

A role for GAL in glutamate-mediated LTP in the hippocampus may also contribute to GAL's effects on memory. Electrophysiological studies in rodent hippocampal slices show that GAL restricts LTP at both perforant path-dentate gyrus and Schaffer collateral-CA1 synapses [9,11,13,30]. GAL may impact glutamatergic transmission in the hippocampus by

reducing evoked glutamate release [11,83–85]. However, while GAL inhibits LTP at CA1 synapses, it has no effect on ionotropic AMPA or NMDA glutamate receptor-mediated EPSPs, suggesting that GAL acts through a postsynaptic GALR to inhibit LTP-related signaling cascades [9].

The development of transgenic mice that overexpress GAL has facilitated the study of GAL overexpression in the brain and provides a unique model for the investigation of this peptide in the area of cognition [14,86]. For example, mice expressing GAL ectopically under control of the dopamine β-hydroxylase promoter (GAL-tg mice) displayed increased GAL fiber density in the basal forebrain and an ~ 3-fold reduction in the number of ChAT-ir septohippocampal neurons in the horizontal limb of the diagonal band [14]. *In situ* hybridization experiments in these GAL-tg mice demonstrated a downregulation of ChAT mRNA per cell within the horizontal limb without a difference in the number of ChAT mRNA-containing neurons in this area [87]. Hence, GAL overexpression in the basal forebrain of GAL-tg mice may selectively reduce the expression of the cholinergic neuron phenotype. Spatial navigation testing with the Morris water task in GAL-tg mice showed a complete lack of selective search on the probe trial at 8, 16 and 24 months of age [14], indicating the GAL-tg mice could not generate a cognitive map of the spatial environment to solve the probe test, the most challenging component of the task [14,87]. As the Morris task requires an intact hippocampus [14], it seems likely that the mechanisms underlying the observed deficits in the GAL-tg mouse include inhibitory neuromodulation by GAL in the hippocampus. Along these lines, GAL expression is increased as much as ~ 4-fold in the hippocampus of GAL-tg compared to wild-type (WT) mice [88], and GAL overexpression in these mice results in reduced *in vivo* ACh release in the ventral hippocampus [89], mimicking results from rats exposed to exogenous GAL administration (see above). Furthermore, hippocampal slices from GAL-tg mice display reduced glutamate release and restricted LTP at perforant path-dentate gyrus synapses compared to WT mice [11].

A transgenic mouse that overexpresses GAL on a platelet-derived growth factor B promoter (GalOE mice) demonstrated an ~4-fold increase in hippocampal GAL and reduced frequency facilitation of field EPSPs – a form of short-term synaptic plasticity – at mossy fiber-CA3 synapses in GalOE hippocampal slices [86]. Aged GalOE mice also display deficits in paired-pulse facilitation of field EPSP at perforant path-dentate gyrus synapses [90]. Similar to GAL-tg mice, the GalOE mice exhibited age-dependent impairments on the Morris water maze [91] possibly related to septal cholinergic function, as behavioral impairment was concomitant with decreased hippocampal ChAT activity [91]. Taken together, these data suggest that GAL overexpression in mouse models inhibits multiple neurotransmitter systems involved in cognitive function. Since the organization of the galaninergic basal forebrain and LC systems differ between rodents and humans [73,79,92], it is an open question as to whether the physiological actions seen in GAL transgenic mice or rat studies translate to the human condition.

Galanin plasticity as a neuroprotective factor in AD

An alternative hypothesis to the neuroinhibitory action of GAL is that overexpression of the peptide is neuroprotective in AD. With respect to the cholinergic NB, it is intriguing to note that GAL hyperinnervation and GAL binding sites are greatest in the anterior NB subfield in AD where the least amount of neural degeneration occurs [27]. By contrast, there is no evidence for GAL fiber [22,23] or GALR [26,27] overexpression within the posterior NB subfields where cholinergic neuron degeneration is greatest [93,94]. In addition to the custom cDNA array data presented above showing reduced expression of PP subunits in single cholinergic NB neurons hyperinnervated by GAL, which potentially slows NFT formation, we have also used this technique to show that ChAT mRNA levels are selectively

increased in GAL-hyperinnervated NB neurons in AD compared to non-innervated NB neurons in control or AD brains [34] (Fig. 3). These observations from postmortem AD tissue offer evidence that GAL overexpression upon NB neurons may result in the protection of cholinergic neuron function as the disease progresses.

A role for GAL in cholinergic cell survival was demonstrated in a knockout mouse model carrying a targeted loss-of-function mutation in the GAL gene (GAL-KO mice) [13,95]. GAL-KO mice showed a significant decrease in the number of ChAT-ir neurons in the CBF medial septum and vertical limb/diagonal band subfields. Moreover, these areas as well as the NB displayed a significant decrease in the number of neurons expressing TrkA, the high-affinity receptor for the cholinergic cell survival substance nerve growth factor (NGF) [13]. GAL-KO mice exhibited an age-related decrease in evoked ACh release in the hippocampus, inhibition of LTP in the CA1 region of the hippocampus, and age-dependent behavioral decline on the Morris water maze [13] and object-in-place [96] spatial memory tasks, indicating an excitatory role for GAL in the hippocampal function of these mutant mice. Significantly, electrophysiological studies using rat primary CBF diagonal band neuron cultures showed that exogenous GAL reduced an array of inhibitory potassium currents in cholinergic neurons [97]. GAL also increased the excitability of these cells under current-clamp conditions [97]. These results complement *in vivo* studies showing that chronic infusion of 1–3 nanomolar GAL into the rat medial septum/diagonal band region resulted in increased ACh release in the ventral hippocampus and improved spatial memory performance on the water maze [98]. This finding from awake, freely moving animals stands in contrast to the inhibitory effects of GAL on ACh release described in rat hippocampal slices (see above) and suggests that a putative neuroprotective role for GAL may involve the survival and/or regulation of the tone of CBF neurons. In support of this hypothesis, GAL has been shown to protect rat CBF septal neuron cultures from Aβ neurotoxicity by increasing pro-survival signaling (e.g., via phosphorylated Akt) and reducing apoptotic signaling (e.g., caspase 3 cleavage) [33]. Intriguingly, the GALR2 agonist AR-M1896 mimicked GAL in this paradigm, suggesting the involvement of this receptor in mediating the neuroprotective effects of GAL [33].

Several lines of evidence indicate that activation of the GALR2 receptor elicits a neuroprotective signal. The AR-M1896 GALR2 agonist protects both mouse [32] and rat [99] primary neuronal hippocampal cultures from glutamate or staurosporine-induced [32] cell death. More recently, GAL failed to prevent glutamate-induced hippocampal cell death in cultures from GALR2 knockout (GALR2KO) mice [100]. Moreover, GAL stimulation of the neuroprotective Akt and Erk phosphorylation signaling cascades in hippocampal cultures from WT mice was significantly attenuated in GALR2KO cultures, suggesting that GAL neuroprotection involved GALR2-mediated stimulation of these pathways [100]. Supporting this notion is the observation that GAL and the GALR2-preferring GAL-like peptide induce neurite outgrowth in PC12 cells in an Erk-dependent manner [101].

The conflicting data regarding the putative functional consequences of GAL overexpression in AD have yet to be reconciled, especially in light of the similar detrimental phenotypes observed in the GAL-KO and GAL-tg/GalOE mice. With respect to the CBF, results from the GAL-KO mouse suggest that GAL is important for the establishment of cholinergic basocortical and septohippocampal systems [13]. The hypertrophy of GAL systems in AD may be an attempt by the brain to replicate developmental actions of this peptide in response to the degeneration of CBF cortical and hippocampal projection neurons. Interestingly, GAL and its receptors are expressed in embryonic stem cells, suggesting that GAL may be a crucial factor in cell differentiation/survival during embryogenesis [102]. Data from human tissue studies and *in vivo* and *in vitro* rodent models indicate a potential neuroprotective effect of GAL plasticity upon CBF neurons. However, GAL plasticity within the

hippocampus may inhibit cholinergic transmission, as inferred from the phenotype of the GAL-tg/GalOE mice and rodent hippocampal preparations. Hence, GAL may induce a neuroprotective signal in the somatodendritic compartment but may play an inhibitory role in the axonal compartment of CBF neurons vulnerable to AD pathophysiology [103]. Given the diverse repertoire of context-dependent GALR signaling pathways, e.g., GALR1 inhibits adenylyl cyclase or activates Erk, GALR2 activates phospholipase C and activates or inhibits adenylyl cyclase [100,104,105], delineating the GALR(s) activated by GAL in the human CBF and hippocampus will be critical for clarifying the functional effects of GAL in AD. Knockout mice deficient for GALR subtypes may ultimately help clarify the role(s) of GAL signaling in cognitive processes. However, findings from GALR1 knockout (GALR1KO) and GALR2KO mice have not yielded definitive results. For instance, whereas studies in GALR1KO mice demonstrated the requirement of this receptor for neuroprotective, anticonvulsant effects in hippocampus in the face of spontaneous or experimentally induced seizures [106–108], pharmacological [109] and molecular [110] manipulations also implicated GALR2 activity in these activities. Likewise, studies of GALR1KO and GALR2KO mouse strains have implicated both receptors in the mediation of anxiolytic actions [111,112]. With respect to learning and memory tasks, the GALR1KO mouse displayed a significant impairment in a fear conditioning emotional memory task [112], linking this receptor to cognitive processes mediated by the amygdala.On the other hand, neither GALR knockout model exhibited deficits in spatial memory tasks [112,113]. These surprising findings suggest that GAL effects on learning and memory may involve a complex interplay between GALR1 and GALR2 receptors, perhaps in pre- and postsynaptic compartments of CBF and hippocampal neurons, or that the more sparsely distributed GALR3 receptor is also involved in the expression of these cognitive processes.

Galanin receptors as therapeuric targets for AD

Currently approved drug treatments for AD include cholinesterase inhibitors, which act by increasing the bioavailability of synaptic ACh [114], and memantine, a noncompetitive glutamatergic NMDA receptor antagonist that suppresses excitotoxicity [115]. These drugs produce small but consistent improvements of memory and global function and positively influence activities of daily living [114,115]. If GAL inhibits ACh release, then GALR subtype-specific antagonists may enhance cholinergic transmission by reducing the inhibitory influence of GAL on the firing rate of CBF neurons. Likewise, if GAL promotes the survival or cholinergic tone of CBF neurons, then a GALR agonist might prove efficacious. Intriguingly, gene expression profiling studies revealed that human CBF neurons express mRNAs encoding all three GALRs [38]; hence, the predominant GAL-mediated signal elicited in innervated and hyperinnervated cholinergic neurons is unclear. Moreover, unlike human CBF neurons, very few rodent CBF neurons express GALR1 [92], so this will present a potential confound in extrapolating results from animal models of cognition to humans. The ambiguous results from GALRKO mice with respect to rodent memory tasks analogous to human working memory function suggest that *in vivo* GALR subtype-specific pharmacological manipulations can potentially clarify the role of each GALR in the face of basal and augmented GAL signaling. Until recently, the only tools available for pharmacological differentiation of GALR subtypes have been synthetic GAL analogs with one or more amino acid substitution or chimeric GAL peptide ligands that show variable affinity for human and rat GALRs and incongruently behave as antagonists at native receptors but as partial or weak agonists at cloned receptors [84,116–120]. However, two peptidergic compounds, AR-M1896 and AR-M961, which are selective agonists for GALR2 and GALR1/GALR2, respectively [121], have been used in rat models to identify GALR subtype specific activities in nociception (mediated by GALR2) and analgesia (GALR1) in the spinal cord [121], hyperpolarization of LC neurons (GALR1; [5]), and neuritogenesis in cholinergic sensory neuron explants (GALR2; [122]). More recently, the

non-peptidergic GALR1 agonist galmic was discovered and mimics GAL in suppressing LTP and seizures in the rodent hippocampus [123]. The effects of these ligands on cognitive function have not been firmly established. The ongoing search for selective GALR ligands in drug discovery programs will hopefully provide new research tools needed to understand GALR subtype-specific pharmacology with respect to cognitive processes mediated by neuronal populations vulnerable to AD pathogenesis. Since AD appears to arise from multiple etiologies, a rational treatment strategy might include high-affinity GALR ligands used in combination with anticholinesterases and perhaps other compounds, such as memantine and modulators of Aβ aggregation or clearance. In this regard, intraventricular infusion of NGF increased GAL mRNA expression in the hippocampus of rats [124] suggesting that the use of NGF for the treatment of AD [125] may indirectly increase GAL in the brain, providing a dual therapeutic benefit for treatment of CBF dysfunction in AD. We suggest that the polypharmacological use of such compounds may ameliorate cholinergic hypofunction in AD and perhaps benefit other aspects of this heterogeneous disorder.

Conclusions

The presentation of AD is likely precipitated by neuronal degeneration in selectively vulnerable regions of the limbic system and brainstem involved in higher-order cognitive processes. Findings presented in this review reveal that GAL has important cell survival actions (see also the review by Elliott-Hunt and Wynick in this issue) upon several neurotransmitter systems and raises the intriguing possibility that pharmacological manipulation of GAL activity might be neuroprotective and slow cognitive decline in AD. This concept deviates from the traditional hypothesis that GAL inhibits neuronal systems related to cognition in the rodent brain. Since there is a major species difference in the expression of GAL within the CBF and LC of the human and rodent brain [73,78,79,92], the physiological actions of GAL may also have differentiated during the evolution of the human brain, where GAL may be organized to fine-tune the functional tone of select neuronal populations such as those involved in learning and memory [126]. Therefore, the functional consequences of GAL plasticity in AD must be clarified to guide the development of high-affinity GALR subtype-specific agonists or antagonists. Future elucidation of GALR distribution through the development of subtype-specific antibodies and of endogenous GALR activity through the continued development of subtype-specific ligands and transgenic GALR mice will be critical for gauging the therapeutic efficacy of GAL mimetics for the amelioration of AD symptoms.

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References

1. Dutar P, Lamour Y, Nicoll RA. Galanin blocks the slow cholinergic EPSP in CA1 pyramidal neurons from ventral hippocampus. Eur. J. Pharmacol 1989;164:355–360. [PubMed: 2474449]
2. Fisone G, Wu CF, Consolo S, Nordstrom O, Brynne N, Bartfai T, Melander T, Hokfelt T. Galanin inhibits acetylcholine release in the ventral hippocampus of the rat: histochemical, autoradiographic, in vivo, and in vitro studies. Proc. Natl. Acad. Sci. USA 1987;84:7339–7343. [PubMed: 2444980]

3. Kehr J, Yoshitake T, Wang FH, Razani H, Gimenez-Llort L, Jansson A, Yamaguchi M, Ogren SO. Galanin is a potent in vivo modulator of mesencephalic serotonergic neurotransmission. Neuropsychopharmacology 2002;27:341–356. [PubMed: 12225692]
4. Levin MC, Sawchenko PE, Howe PR, Bloom SR, Polak JM. Organization of galanin-immunoreactive inputs to the paraventricular nucleus with special reference to their relationship to catecholaminergic afferents. J. Comp. Neurol 1987;261:562–582. [PubMed: 2440918]
5. Ma X, Tong YG, Schmidt R, Brown W, Payza K, Hodzic L, Pou C, Godbout C, Hokfelt T, Xu ZQ. Effects of galanin receptor agonists on locus coeruleus neurons. Brain Res 2001;919:169–174. [PubMed: 11689176]
6. Merchenthaler I. The hypophysiotropic galanin system of the rat brain. Neuroscience 1991;44:643–654. [PubMed: 1721685]
7. Pieribone VA, Xu ZQ, Zhang X, Grillner S, Bartfai T, Hokfelt T. Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. Neuroscience 1995;64:861–874. [PubMed: 7538638]
8. Xu ZQ, Zhang X, Pieribone VA, Grillner S, Hokfelt T. Galanin-5-hydroxytryptamine interactions: electrophysiological, immunohistochemical and in situ hybridization studies on rat dorsal raphe neurons with a note on galanin R1 and R2 receptors. Neuroscience 1998;87:79–94. [PubMed: 9722143]
9. Coumis U, Davies CH. The effects of galanin on long-term synaptic plasticity in the CA1 area of rodent hippocampus. Neuroscience 2002;112:173–182. [PubMed: 12044482]
10. Crawley JN. Minireview. Galanin-acetylcholine interactions: relevance to memory and Alzheimer's disease. Life Sci 1996;58:2185–2199. [PubMed: 8649205]
11. Mazarati AM, Hohmann JG, Bacon A, Liu H, Sankar R, Steiner RA, Wynick D, Wasterlain CG. Modulation of hippocampal excitability and seizures by galanin. J. Neurosci 2000;20:6276–6281. [PubMed: 10934278]
12. Ogren SO, Hokfelt T, Kask K, Langel U, Bartfai T. Evidence for a role of the neuropeptide galanin in spatial learning. Neuroscience 1992;51:1–5. [PubMed: 1281521]
13. O'Meara G, Coumis U, Ma SY, Kehr J, Mahoney S, Bacon A, Allen SJ, Holmes F, Kahl U, Wang FH, et al. Galanin regulates the postnatal survival of a subset of basal forebrain cholinergic neurons. Proc. Natl. Acad. Sci. USA 2000;97:11569–11574. [PubMed: 11016971]
14. Steiner RA, Hohmann JG, Holmes A, Wrenn CC, Cadd G, Jureus A, Clifton DK, Luo M, Gutshall M, Ma SY, et al. Galanin transgenic mice display cognitive and neurochemical deficits characteristic of Alzheimer's disease. Proc. Natl. Acad. Sci. USA 2001;98:4184–4189. [PubMed: 11259657]
15. Wrenn CC, Crawley JN. Pharmacological evidence supporting a role for galanin in cognition and affect. Prog. Neuropsychopharmacol. Biol. Psychiatry 2001;25:283–299.
16. Mesulam MM, Mufson EJ, Levey AI, Wainer BH. Cholinergic innervation of cortex by the basal fore-brain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. J. Comp. Neurol 1983;214:170–197. [PubMed: 6841683]
17. Bartus RT. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp. Neurol 2000;163:495–529. [PubMed: 10833325]
18. Baxter MG, Chiba AA. Cognitive functions of the basal forebrain. Curr. Opin. Neurobiol 1999;9:178–183. [PubMed: 10322180]
19. Sarter M, Bruno JP, Turchi J. Basal forebrain afferent projections modulating cortical acetylcholine, attention, and implications for neuropsychiatric disorders. Ann. N. Y. Acad. Sci 1999;877:368–382. [PubMed: 10415659]
20. Wilcock GK, Esiri MM, Bowen DM, Smith CC. Alzheimer's disease. Correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. J. Neurol. Sci 1982;57:407–417. [PubMed: 7161627]
21. Bowser R, Kordower JH, Mufson EJ. A confocal microscopic analysis of galaninergic hyperinnervation of cholinergic basal forebrain neurons in Alzheimer's disease. Brain Pathol 1997;7:723–730. [PubMed: 9161723]

22. Chan-Palay V. Galanin hyperinnervates surviving neurons of the human basal nucleus of Meynert in dementias of Alzheimer's and Parkinson's disease: a hypothesis for the role of galanin in accentuating cholinergic dysfunction in dementia. J. Comp. Neurol 1988;273:543–557. [PubMed: 2463283]
23. Mufson EJ, Cochran E, Benzing W, Kordower JH. Galaninergic innervation of the cholinergic vertical limb of the diagonal band (Ch2) and bed nucleus of the stria terminalis in aging, Alzheimer's disease and Down's syndrome. Dementia 1993;4:237–250. [PubMed: 7505157]
24. Bierer LM, Haroutunian V, Gabriel S, Knott PJ, Carlin LS, Purohit DP, Perl DP, Schmeidler J, Kanof P, Davis KL. Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits. J. Neurochem 1995;64:749–760. [PubMed: 7830069]
25. Gabriel SM, Bierer LM, Davidson M, Purohit DP, Perl DP, Harotunian V. Galanin-like immunoreactivity is increased in the postmortem cerebral cortex from patients with Alzheimer's disease. J. Neurochem 1994;62:1516–1523. [PubMed: 7510783]
26. McMillan PJ, Peskind E, Raskind MA, Leverenz JB. Increased galanin receptor occupancy in Alzheimer's disease. Neurobiol. Aging 2004;25:1309–1314. [PubMed: 15465628]
27. Mufson EJ, Deecher DC, Basile M, Izenwasse S, Mash DC. Galanin receptor plasticity within the nucleus basalis in early and late Alzheimer's disease: an in vitro autoradiographic analysis. Neuropharmacology 2000;39:1404–1412. [PubMed: 10818256]
28. Perez S, Basile M, Mash DC, Mufson EJ. Galanin receptor over-expression within the amygdala in early Alzheimer's disease: an in vitro autoradiographic analysis. J. Chem. Neuroanat 2002;24:109–116. [PubMed: 12191727]
29. Rodriguez-Puertas R, Nilsson S, Pascual J, Pazos A, Hokfelt T. 125I–galanin binding sites in Alzheimer's disease: increases in hippocampal subfields and a decrease in the caudate nucleus. J. Neurochem 1997;68:1106–1113. [PubMed: 9048756]
30. Sakurai E, Maeda T, Kaneko S, Akaike A, Satoh M. Galanin inhibits long-term potentiation at Schaffer collateral-CA1 synapses in guinea-pig hippocampal slices. Neurosci. Lett 1996;212:21–24. [PubMed: 8823753]
31. McDonald MP, Gleason TC, Robinson JK, Crawley JN. Galanin inhibits performance on rodent memory tasks. Ann. N. Y. Acad. Sci 1998;863:305–322. [PubMed: 9928180]
32. Elliott-Hunt CR, Marsh B, Bacon A, Pope R, Vanderplank P, Wynick D. Galanin acts as a neuroprotective factor to the hippocampus. Proc. Natl. Acad. Sci. USA 2004;101:5105–5110. [PubMed: 15041741]
33. Ding X, MacTavish D, Kar S, Jhamandas JH. Galanin attenuates beta-amyloid (Abeta) toxicity in rat cholinergic basal forebrain neurons. Neurobiol. Dis 2006;21:413–420. [PubMed: 16246567]
34. Counts SE, He B, Che S, Ginsberg SD, Mufson EJ. Galanin hyperinnervation upregulates choline acetyl-transferase expression in cholinergic basal forebrain neurons in Alzheimer's disease. Neurodegener. Dis 2008;5:228–231. [PubMed: 18322398]
35. Counts SE, Perez SE, Ginsberg SD, De Lacalle S, Mufson EJ. Galanin in Alzheimer disease. Mol. Interv 2003;3:137–156. [PubMed: 14993421]
36. Beal MF, MacGarvey U, Swartz KJ. Galanin immunoreactivity is increased in the nucleus basalis of Meynert in Alzheimer's disease. Ann. Neurol 1990;28:157–161. [PubMed: 1699471]
37. Petersen RC. Mild cognitive impairment as a diagnostic entity. J. Intern. Med 2004;256:183–194. [PubMed: 15324362]
38. Counts SE, Chen EY, Che S, Ikonomovic MD, Wuu J, Ginsberg SD, Dekosky ST, Mufson EJ. Galanin fiber hypertrophy within the cholinergic nucleus basalis during the progression of Alzheimer's disease. Dement. Geriatr. Cogn. Disord 2006;21:205–214. [PubMed: 16410678]
39. Chamberlain SR, Muller U, Blackwell AD, Robbins TW, Sahakian BJ. Noradrenergic modulation of working memory and emotional memory in humans. Psychopharmacology (Berl.) 2006;188:397–407. [PubMed: 16642355]
40. Zarow C, Lyness SA, Mortimer JA, Chui HC. Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. Arch. Neurol 2003;60:337–341. [PubMed: 12633144]

41. Chan-Palay V. Alterations in the locus coeruleus in dementias of Alzheimer's and Parkinson's disease. Prog. Brain Res 1991;88:625–630. [PubMed: 1726030]
42. Deecher DC, Mash DC, Staley JK, Mufson EJ. Characterization and localization of galanin receptors in human entorhinal cortex. Regul. Pept 1998;73:149–159. [PubMed: 9556077]
43. Kohler C, Chan-Palay V. Galanin receptors in the post-mortem human brain. Regional distribution of 125I–galanin binding sites using the method of in vitro receptor autoradiography. Neurosci. Lett 1990;120:179–182. [PubMed: 1705678]
44. Amaral, DG. Memory: anatomical organization of candidate brain regions. In: Mountcastle, VB.; Plum, F.; Geiger, SR., editors. Handbook of Physiology. Section 1: The Nervous System. V. Higher Functions of the Brain. Vol. part 1. Bethesda, MD: American Physiological Society; 1987. p. 211-294.
45. Gomez-Isla T, Price JL, McKeel DW Jr, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. J. Neurosci 1996;16:4491–4500. [PubMed: 8699259]
46. Kordower JH, Chu Y, Stebbins GT, DeKosky ST, Cochran EJ, Bennett D, Mufson EJ. Loss and atrophy of layer II entorhinal cortex neurons in elderly people with mild cognitive impairment. Ann. Neurol 2001;49:202–213. [PubMed: 11220740]
47. Amaral, DG. Amygdalohippocampal and amygdalocortical projections in the primate brain. In: Schwartz, R.; Ben-Ari, Y., editors. Excitatory Amino Acids and Epilepsy. Vol. vol. 203. New York: Plenum Press; 1986. p. 3-17.
48. Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A. Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Ann. N. Y. Acad. Sci 2000;911:369–391. [PubMed: 10911886]
49. Vereecken TH, Vogels OJ, Nieuwenhuys R. Neuron loss and shrinkage in the amygdala in Alzheimer's disease. Neurobiol. Aging 1994;15:45–54. [PubMed: 8159262]
50. Holmes PV, Crawley JN. Olfactory bulbectomy increases prepro-galanin mRNA levels in the rat locus coeruleus. Brain Res. Mol. Brain Res 1996;36:184–188. [PubMed: 9011757]
51. Villar MJ, Meister B, Hokfelt T. Reorganization of neural peptidergic systems in the median eminence after hypophysectomy. J. Neurosci 1994;14:5996–6012. [PubMed: 7523631]
52. Gabriel SM, Knott PJ, Haroutunian V. Alterations in cerebral cortical galanin concentrations following neurotransmitter-specific subcortical lesions in the rat. J. Neurosci 1995;15:5526–5534. [PubMed: 7543947]
53. Hartonian I, Mufson EJ, de Lacalle S. Long-term plastic changes in galanin innervation in the rat basal forebrain. Neuroscience 2002;115:787–795. [PubMed: 12435417]
54. Shen PJ, Larm JA, Gundlach AL. Expression and plasticity of galanin systems in cortical neurons, oligodendrocyte progenitors and proliferative zones in normal brain and after spreading depression. Eur. J. Neurosci 2003;18:1362–1376. [PubMed: 14511317]
55. Bond BC, Virley DJ, Cairns NJ, Hunter AJ, Moore GB, Moss SJ, Mudge AW, Walsh FS, Jazin E, Preece P. The quantification of gene expression in an animal model of brain ischaemia using TaqMan real-time RT-PCR. Brain Res. Mol. Brain Res 2002;106:101–116. [PubMed: 12393270]
56. Villar MJ, Cortes R, Theodorsson E, Wiesenfeld-Hallin Z, Schalling M, Fahrenkrug J, Emson PC, Hokfelt T. Neuropeptide expression in rat dorsal root ganglion cells and spinal cord after peripheral nerve injury with special reference to galanin. Neuroscience 1989;33:587–604. [PubMed: 2484006]
57. Bacon A, Holmes FE, Small CJ, Ghatei M, Mahoney S, Bloom S, Wynick D. Transgenic overexpression of galanin in injured primary sensory neurons. Neuroreport 2002;13:2129–2132. [PubMed: 12438940]
58. Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun 1984;120:885–890. [PubMed: 6375662]
59. Kowall NW, Beal MF. Galanin-like immunoreactivity is present in human substantia innominata and in senile plaques in Alzheimer's disease. Neurosci. Lett 1989;98:118–123. [PubMed: 2469045]

60. Diez M, Koistinaho J, Kahn K, Games D, Hokfelt T. Neuropeptides in hippocampus and cortex in transgenic mice overexpressing V717F beta-amyloid precursor protein-initial observations. Neuroscience 2000;100:259–286. [PubMed: 11008166]
61. Diez M, Danner S, Frey P, Sommer B, Staufenbiel M, Wiederhold KH, Hokfelt T. 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 2003;14:579–594. [PubMed: 14678773]
62. Lazarov O, Lee M, Peterson DA, Sisodia SS. Evidence that synaptically released beta-amyloid accumulates as extracellular deposits in the hippocampus of transgenic mice. J. Neurosci 2002;22:9785–9793. [PubMed: 12427834]
63. Perez SE, Dar S, Ikonomovic MD, DeKosky ST, Mufson EJ. Cholinergic forebrain degeneration in the APPswe/PS1DeltaE9 transgenic mouse. Neurobiol. Dis 2007;28:3–15. [PubMed: 17662610]
64. Perez SE, Lazarov O, Koprich JB, Chen EY, Rodriguez-Menendez V, Lipton JW, Sisodia SS, Mufson EJ. Nigrostriatal dysfunction in familial Alzheimer's disease-linked APPswe/PS1DeltaE9 transgenic mice. J. Neurosci 2005;25:10220–10229. [PubMed: 16267229]
65. Arendt T, Taubert G, Bigl V, Arendt A. Amyloid deposition in the nucleus basalis of Meynert complex: a topographic marker for degenerating cell clusters in Alzheimer's disease. Acta Neuropathol. (Berl.) 1988;75:226–232. [PubMed: 3348080]
66. Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 1992;42:631–639. [PubMed: 1549228]
67. Kosik KS, Joachim CL, Selkoe DJ. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc. Natl. Acad. Sci. USA 1986;83:4044–4048. [PubMed: 2424016]
68. Wood JG, Mirra SS, Pollock NJ, Binder LI. Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau. Proc. Natl. Acad. Sci. USA 1986;83:4040–4043. [PubMed: 2424015]
69. Guillozet-Bongaarts AL, Garcia-Sierra F, Reynolds MR, Horowitz PM, Fu Y, Wang T, Cahill ME, Bigio EH, Berry RW, Binder LI. Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease. Neurobiol. Aging 2005;26:1015–1022. [PubMed: 15748781]
70. Mufson EJ, Counts SE, Perez SE, Binder L. Galanin plasticity in the cholinergic basal forebrain in Alzheimer's disease and transgenic mice. Neuropeptides 2005;39:232–236.
71. Trojanowski JQ, Lee VM. Phosphorylation of paired helical filament tau in Alzheimer's disease neurofibrillary lesions: focusing on phosphatases. FASEB J 1995;9:1570–1576. [PubMed: 8529836]
72. Chan-Palay V. Neurons with galanin innervate cholinergic cells in the human basal forebrain and galanin and acetylcholine coexist. Brain Res. Bull 1988;21:465–472. [PubMed: 2463858]
73. Kordower JH, Mufson EJ. Galanin-like immunoreactivity within the primate basal forebrain: differential staining patterns between humans and monkeys. J. Comp. Neurol 1990;294:281–292. [PubMed: 1692044]
74. Holets VR, Hokfelt T, Rokaeus A, Terenius L, Goldstein M. Locus coeruleus neurons in the rat containing neuropeptide Y, tyrosine hydroxylase or galanin and their efferent projections to the spinal cord, cerebral cortex and hypothalamus. Neuroscience 1988;24:893–906. [PubMed: 2454419]
75. Miller MA, Kolb PE, Leverenz JB, Peskind ER, Raskind MA. Preservation of noradrenergic neurons in the locus ceruleus that coexpress galanin mRNA in Alzheimer's disease. J. Neurochem 1999;73:2028–2036. [PubMed: 10537062]
76. Xu ZQ, Shi TJ, Hokfelt T. Galanin/GMAP-and NPY-like immunoreactivities in locus coeruleus and noradrenergic nerve terminals in the hippocampal formation and cortex with notes on the galanin-R1 and -R2 receptors. J. Comp. Neurol 1998;392:227–251. [PubMed: 9512271]
77. Nieuwenhuys, R. Chemoarchitecture of the Brain. Berlin: Springer; 1985.
78. Perez SE, Wynick D, Steiner RA, Mufson EJ. Distribution of galaninergic immunoreactivity in the brain of the mouse. J. Comp. Neurol 2001;434:158–185. [PubMed: 11331523]

79. Skofitsch G, Jacobowitz DM. Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. Peptides 1985;6:509–546. [PubMed: 2415952]
80. Palazzi E, Felinska S, Zambelli M, Fisone G, Bartfai T, Consolo S. Galanin reduces carbachol stimulation of phosphoinositide turnover in rat ventral hippocampus by lowering Ca2+ influx through voltage-sensitive Ca2+ channels. J. Neurochem 1991;56:739–747. [PubMed: 1704418]
81. Robinson JK, Zocchi A, Pert A, Crawley JN. Galanin microinjected into the medial septum inhibits scopolamine-induced acetylcholine overflow in the rat ventral hippocampus. Brain Res 1996;709:81–87. [PubMed: 8869559]
82. Robinson JK, Crawley JN. Intraseptal galanin potentiates scopolamine impairment of delayed nonmatching to sample. J. Neurosci 1993;13:5119–5125. [PubMed: 7504722]
83. Ben-Ari Y. Galanin and glibenclamide modulate the anoxic release of Glutamate in rat CA3 hippocampal neurons. Eur. J. Neurosci 1990;2:62–68. [PubMed: 12106103]
84. Kinney GA, Emmerson PJ, Miller RJ. Galanin receptor-mediated inhibition of glutamate release in the arcuate nucleus of the hypothalamus. J. Neurosci 1998;18:3489–3500. [PubMed: 9570780]
85. Zini S, Roisin MP, Langel U, Bartfai T, Ben-Ari Y. Galanin reduces release of endogenous excitatory amino acids in the rat hippocampus. Eur. J. Pharmacol 1993;245:1–7. [PubMed: 7682961]
86. Kokaia M, Holmberg K, Nanobashvili A, Xu ZQ, Kokaia Z, Lendahl U, Hilke S, Theodorsson E, Kahl U, Bartfai T, et al. Suppressed kindling epileptogenesis in mice with ectopic overexpression of galanin. Proc. Natl. Acad. Sci. USA 2001;98:14006–14011. [PubMed: 11698649]
87. Crawley JN, Mufson EJ, Hohmann JG, Teklemichael D, Steiner RA, Holmberg K, Xu ZQ, Blakeman KH, Xu XJ, Wiesenfeld-Hallin Z, et al. Galanin overexpressing transgenic mice. Neuropeptides 2002;36:145–156. [PubMed: 12359505]
88. Wrenn CC, Marriott LK, Kinney JW, Holmes A, Wenk GL, Crawley JN. Galanin peptide levels in hippocampus and cortex of galanin-overexpressing transgenic mice evaluated for cognitive performance. Neuropeptides 2002;36:413–426. [PubMed: 12507436]
89. Laplante F, Crawley JN, Quirion R. Selective reduction in ventral hippocampal acetylcholine release in awake galanin-treated rats and galanin-overexpressing transgenic mice. Regul. Pept 2004;122:91–98. [PubMed: 15380926]
90. Zheng K, Kuteeva E, Xia S, Bartfai T, Hokfelt T, Xu ZQ. Age-related impairments of synaptic plasticity in the lateral perforant path input to the dentate gyrus of galanin overexpressing mice. Neuropeptides 2005;39:259–267. [PubMed: 15944020]
91. Pirondi S, D'Intino G, Gusciglio M, Massella A, Giardino L, Kuteeva E, Ogren SO, Hokfelt T, Calza L. Changes in brain cholinergic markers and spatial learning in old galanin-overexpressing mice. Brain Res 2007;1138:10–20. [PubMed: 17266943]
92. Miller MA, Kolb PE, Planas B, Raskind MA. Few cholinergic neurons in the rat basal forebrain coexpress galanin messenger RNA. J. Comp. Neurol 1998;391:248–258. [PubMed: 9518272]
93. Mufson EJ, Bothwell M, Kordower JH. Loss of nerve growth factor receptor-containing neurons in Alzheimer's disease: a quantitative analysis across subregions of the basal forebrain. Exp. Neurol 1989;105:221–232. [PubMed: 2548888]
94. Vogels OJ, Broere CA, ter Laak J, ten Donkelaar HJ, Nieuwenhuys R, Schulte BP. Cell loss and shrinkage in the nucleus basalis Meynert complex in Alzheimer's disease. Neurobiol. Aging 1990;11:3–13. [PubMed: 2183081]
95. Wynick D, Bacon A. Targeted disruption of galanin: new insights from knock-out studies. Neuropeptides 2002;36:132–144. [PubMed: 12359504]
96. Massey PV, Warburton EC, Wynick D, Brown MW, Bashir ZI. Galanin regulates spatial memory but not visual recognition memory or synaptic plasticity in perirhinal cortex. Neuropharmacology 2003;44:40–48. [PubMed: 12559120]
97. Jhamandas JH, Harris KH, MacTavish D, Jassar BS. Novel excitatory actions of galanin on rat cholinergic basal forebrain neurons: implications for its role in Alzheimer's disease. J. Neurophysiol 2002;87:696–704. [PubMed: 11826038]
98. Elvander E, Ogren SO. Medial septal galanin and acetylcholine: influence on hippocampal acetylcholine and spatial learning. Neuropeptides 2005;39:245–248. [PubMed: 15944017]

99. Pirondi S, Fernandez M, Schmidt R, Hokfelt T, Giardino L, Calza L. The galanin-R2 agonist AR-M1896 reduces glutamate toxicity in primary neural hippocampal cells. J. Neurochem 2005;95:821–833. [PubMed: 16248891]
100. Elliott-Hunt CR, Pope RJ, Vanderplank P, Wynick D. Activation of the galanin receptor 2 (GalR2) protects the hippocampus from neuronal damage. J. Neurochem 2007;100:780–789. [PubMed: 17263796]
101. Hawes JJ, Narasimhaiah R, Picciotto MR. Galanin and galanin-like peptide modulate neurite outgrowth via protein kinase C-mediated activation of extracellular signal-related kinase. Eur. J. Neurosci 2006;23:2937–2946. [PubMed: 16819983]
102. Tarasov KV, Tarasova YS, Crider DG, Anisimov SV, Wobus AM, Boheler KR. Galanin and galanin receptors in embryonic stem cells: accidental or essential? Neuropeptides 2002;36:239–245. [PubMed: 12372696]
103. Hokfelt T. Galanin and its receptors: introduction to the Third International Symposium, San Diego, California, USA, 21–22 October 2004. Neuropeptides 2005;39:125–142. [PubMed: 15908000]
104. Smith KE, Forray C, Walker MW, Jones KA, Tamm JA, Bard J, Branchek TA, Linemeyer DL, Gerald C. Expression cloning of a rat hypothalamic galanin receptor coupled to phosphoinositide turnover. J. Biol. Chem 1997;272:24612–24616. [PubMed: 9305929]
105. Wang S, Hashemi T, Fried S, Clemmons AL, Hawes BE. Differential intracellular signaling of the GalR1 and GalR2 galanin receptor subtypes. Biochemistry 1998;37:6711–6717. [PubMed: 9578554]
106. Jacoby AS, Hort YJ, Constantinescu G, Shine J, Iismaa TP. Critical role for GALR1 galanin receptor in galanin regulation of neuroendocrine function and seizure activity. Brain Res. Mol. Brain Res 2002;107:195–200. [PubMed: 12487125]
107. Mazarati A, Lu X, Shinmei S, Badie-Mahdavi H, Bartfai T. Patterns of seizures, hippocampal injury and neurogenesis in three models of status epilepticus in galanin receptor type 1 (GalR1) knockout mice. Neuroscience 2004;128:431–441. [PubMed: 15350653]
108. McColl CD, Jacoby AS, Shine J, Iismaa TP, Bekkers JM. Galanin receptor-1 knockout mice exhibit spontaneous epilepsy, abnormal EEGs and altered inhibition in the hippocampus. Neuropharmacology 2006;50:209–218. [PubMed: 16243364]
109. Mazarati A, Lundstrom L, Sollenberg U, Shin D, Langel U, Sankar R. Regulation of kindling epileptogenesis by hippocampal galanin type 1 and type 2 receptors: the effects of subtype-selective agonists and the role of G-protein-mediated signaling. J. Pharmacol. Exp. Ther 2006;318:700–708. [PubMed: 16699066]
110. Mazarati A, Lu X, Kilk K, Langel U, Wasterlain C, Bartfai T. Galanin type 2 receptors regulate neuronal survival, susceptibility to seizures and seizure-induced neurogenesis in the dentate gyrus. Eur. J. Neurosci 2004;19:3235–3244. [PubMed: 15217380]
111. Holmes A, Kinney JW, Wrenn CC, Li Q, Yang RJ, Ma L, Vishwanath J, Saavedra MC, Innerfield CE, Jacoby AS, et al. Galanin GAL-R1 receptor null mutant mice display increased anxiety-like behavior specific to the elevated plus-maze. Neuropsychopharmacology 2003;28:1031–1044. [PubMed: 12700679]
112. Wrenn CC, Kinney JW, Marriott LK, Holmes A, Harris AP, Saavedra MC, Starosta G, Innerfield CE, Jacoby AS, Shine J, et al. Learning and memory performance in mice lacking the GAL-R1 subtype of galanin receptor. Eur. J. Neurosci 2004;19:1384–1396. [PubMed: 15016096]
113. Bailey KR, Pavlova MN, Rohde AD, Hohmann JG, Crawley JN. Galanin receptor subtype 2 (GalR2) null mutant mice display an anxiogenic-like phenotype specific to the elevated plus-maze. Pharmacol. Biochem. Behav 2007;86:8–20. [PubMed: 17257664]
114. Davis KL, Thal LJ, Gamzu ER, Davis CS, Woolson RF, Gracon SI, Drachman DA, Schneider LS, Whitehouse PJ, Hoover TM, et al. A double-blind, placebo-controlled multicenter study of tacrine for Alzheimer's disease. The Tacrine Collaborative Study Group. N. Engl. J. Med 1992;327:1253–1259. [PubMed: 1406817]
115. Lipton SA. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nat. Rev. Drug Discov 2006;5:160–170. [PubMed: 16424917]

116. Bartfai T, Langel U, Bedecs K, Andell S, Land T, Gregersen S, Ahren B, Girotti P, Consolo S, Corwin R, et al. Galanin-receptor ligand M40 peptide distinguishes between putative galanin-receptor subtypes. Proc. Natl. Acad. Sci. USA 1993;90:11287–11291. [PubMed: 7504301]

117. Branchek TA, Smith KE, Gerald C, Walker MW. Galanin receptor subtypes. Trends Pharmacol. Sci 2000;21:109–117. [PubMed: 10689365]

118. Crawley JN, Robinson JK, Langel U, Bartfai T. Galanin receptor antagonists M40 and C7 block galanin-induced feeding. Brain Res 1993;600:268–272. [PubMed: 7679604]

119. Floren A, Land T, Langel U. Galanin receptor subtypes and ligand binding. Neuropeptides 2000;34:331–337. [PubMed: 11162289]

120. McDonald MP, Willard LB, Wenk GL, Crawley JN. Coadministration of galanin antagonist M40 with a muscarinic M1 agonist improves delayed nonmatching to position choice accuracy in rats with cholinergic lesions. J. Neurosci 1998;18:5078–5085. [PubMed: 9634573]

121. Liu HX, Brumovsky P, Schmidt R, Brown W, Payza K, Hodzic L, Pou C, Godbout C, Hokfelt T. Receptor subtype-specific pronociceptive and analgesic actions of galanin in the spinal cord: selective actions via GalR1 and GalR2 receptors. Proc. Natl. Acad. Sci. USA 2001;98:9960–9964. [PubMed: 11481429]

122. Mahoney SA, Hosking R, Farrant S, Holmes FE, Jacoby AS, Shine J, Iismaa TP, Scott MK, Schmidt R, Wynick D. The second galanin receptor GalR2 plays a key role in neurite outgrowth from adult sensory neurons. J. Neurosci 2003;23:416–421. [PubMed: 12533601]

123. Bartfai T, Lu X, Badie-Mahdavi H, Barr AM, Mazarati A, Hua XY, Yaksh T, Haberhauer G, Ceide SC, Trembleau L, Somogyi L, et ak. Galmic, a non-peptide galanin receptor agonist, affects behaviors in seizure, pain, and forced-swim tests. Proc. Natl. Acad. Sci. USA 2004;101:10470–10475. [PubMed: 15240875]

124. Planas B, Kolb PE, Raskind MA, Miller MA. Nerve growth factor induces galanin gene expression in the rat basal forebrain: implications for the treatment of cholinergic dysfunction. J. Comp. Neurol 1997;379:563–570. [PubMed: 9067843]

125. Tuszynski MH, Thal L, Pay M, Salmon DP, U HS, Bakay R, Patel P, Blesch A, Vahlsing HL, Ho G, et al. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. Nat. Med 2005;11:551–555. [PubMed: 15852017]

126. Counts SE, Perez SE, Kahl U, Bartfai T, Bowser RP, Deecher DC, Mash DC, Crawley JN, Mufson EJ. Galanin: neurobiologic mechanisms and therapeutic potential for Alzheimer's disease. CNS Drug Rev 2001;7:445–470. [PubMed: 11830760]

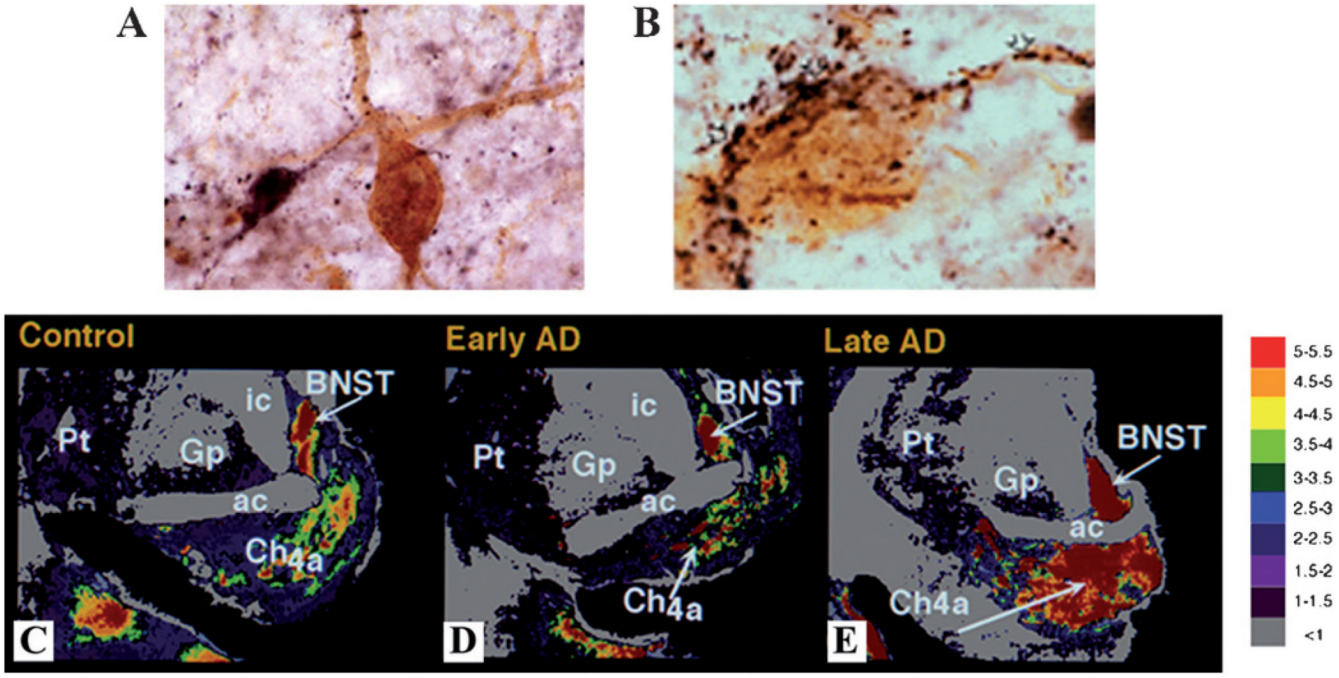

Figure 1.

GAL plasticity in the basal forebrain nucleus basalis in AD. (*A*) Photomicrograph shows a magnocellular cholinergic NB neuron immunostained with the CBF neuronal marker $p75^{NTR}$ (brown reaction product) and innervated by GAL-ir fibers (dark blue reaction product) in aged control brain. Note the GAL-ir parvicellular neuron contacting the CBF neuron. (*B*) Photomicrograph shows a striking hyperinnervation of GAL fibers impinging upon a cholinergic NB neuron in AD. (*C–E*) Pseudocolor density maps showing the regional distribution of [^{125}I]hGAL binding sites in the aged control NB (*C*) as compared to early stage (*D*) and late stage (*E*) AD. Note the increase in labeling in the anterior subfield of the NB in late AD. Gray to red on the color scale indicates increasing GAL binding levels. AD, Alzheimer's disease; NB, nucleus basalis; CBF, cholinergic basal forebrain. ac, anterior comminsure; BSNT, bed nucleus of stria terminalis; Ch4a, NB anterior subfield; GP, globus pallidus; ic, internal capsule; PT, putamen.

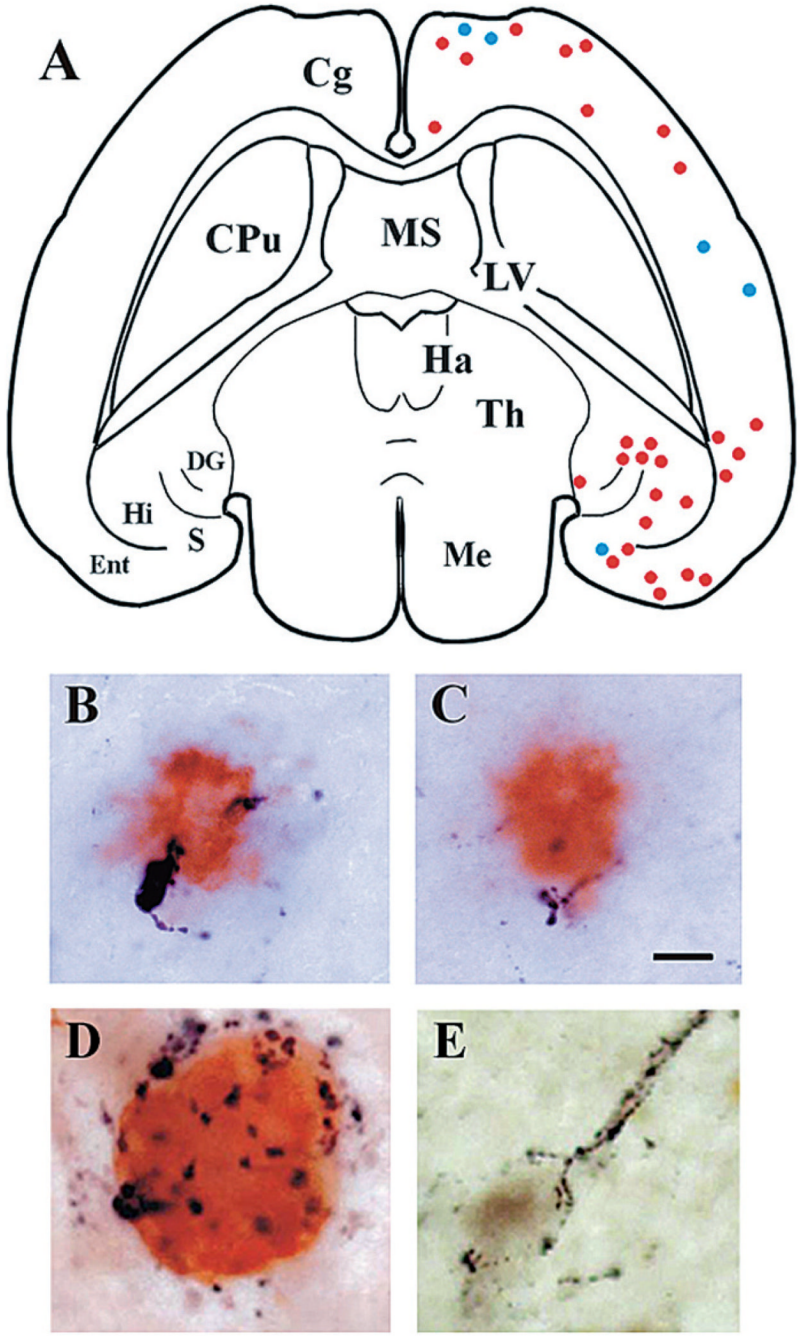

Figure 2.

Association of GAL with AD-related lesions. (*A*) Schematic drawing of a horizontal brain section from a 3-month-old APPswe/PS1ΔE9 transgenic mouse showing the distribution of Aβ-ir plaques with adjacent GAL-ir dystrophic neurites (blue dots) or without dystrophic neurites (red dots). Cg, cingulate cortex; CPu, caudate-putamen; DG, dentate gyrus; Ent, entorhinal cortex; Ha, habenula; Hi, hippocampus; LV, lateral ventricle; Me, mesencephalon; S, subiculum; Th, thalamus. (*B*, *C*) Photomicrographs show co-localization of GAL fibers (black) and Aβ (orange) in amyloid plaques located in the cortex (*B*) and hippocampus (*C*) of a 3-month-old APPswe/PS1ΔE9 mouse. Scale bar, 10 μm. (*D*) GAL hyperinnervation (black) of a cholinergic NB neuron dual-stained for Tau C3 (orange), a tau

epitope that appears early in the evolution of NFTs. (*E*) GAL hyperinnervation (black) upon a cholinergic NB neuron immunonegative for MN423, a late-stage tau event in NFT formation. There was no evidence for GAL hyperinnervation of MN423-immunopositive CBF neurons.

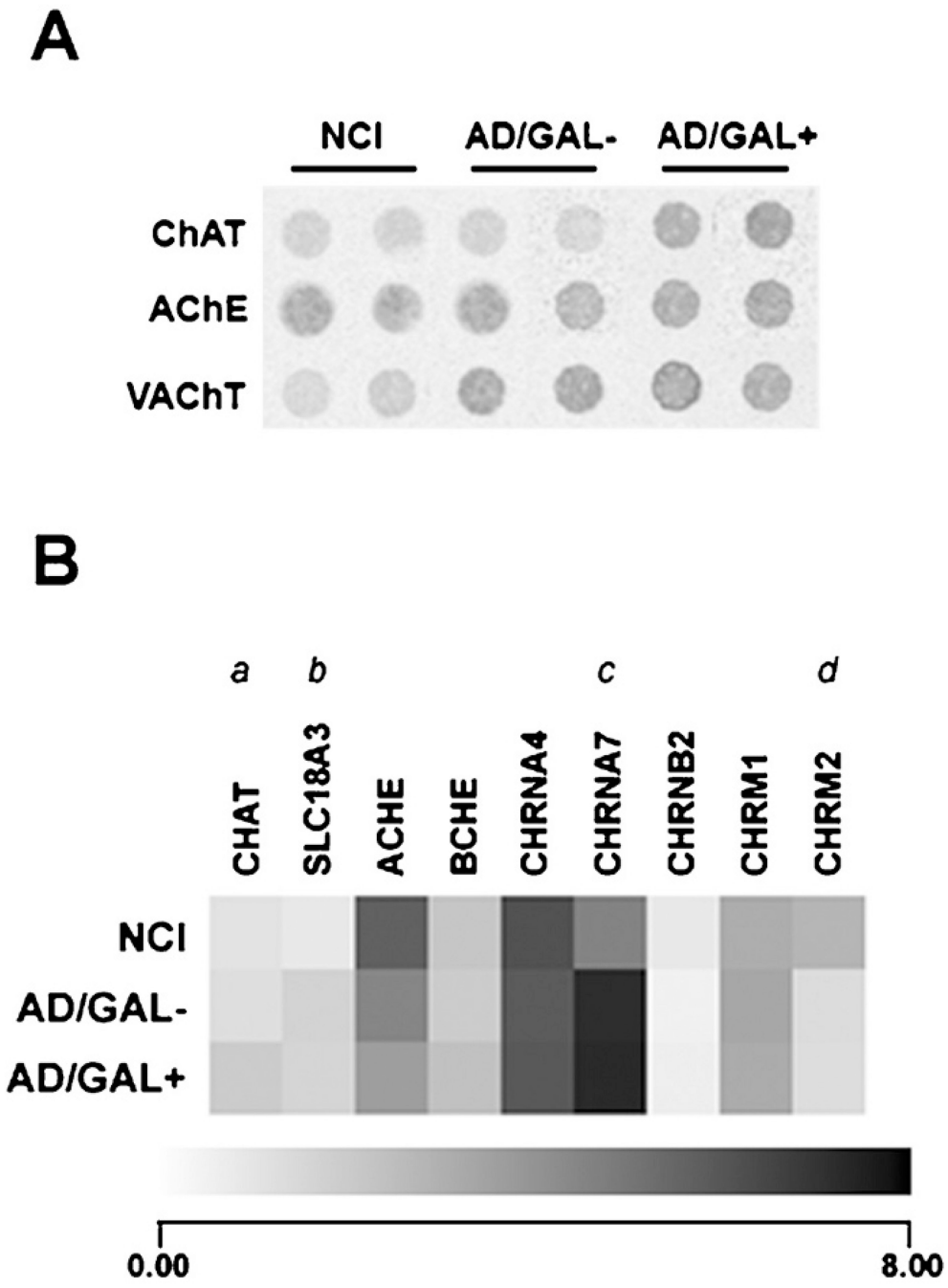

Figure 3.

GAL hyperinnervation upregulates ChAT mRNA levels in single cholinergic NB neurons in AD. (*A*) Representative custom-designed microarray expression data showing relative hybridization signal intensities for ChAT, acetylcholinesterase (AChE), and the vesicular ACh transporter (VAChT) in non-GAL-innervated cholinergic NB neurons from control subjects with no cognitive impairment (NCI), non-GAL-innervated cholinergic NB neurons from AD subjects (AD/GAL−), and GAL-hyperinnervated cholinergic NB neurons from AD subjects (AD/GAL+). (*B*) Dendrogram illustrating relative mRNA expression levels (white to black = increasing levels) of ChAT (Unigene/NCBI notation CHAT), VAChT (SLC18A3), AChE (ACHE), butylcholinesterase (BCHE), nicotinic ACh receptor subunits

α4 (CHRNA4), α7 (CHRNA7), β2 (CHRNB2), and muscarinic ACh receptor subtypes M1 (CHRM1) and M2 (CHRM2). *a* = AD/GAL+>NCI, AD/GAL−, $p<0.01$; *b* = AD/GAL+, AD/GAL− >NCI, $p < 0.01$; *c* = AD/GAL+, AD/GAL− >NCI, $p < 0.001$; *d* = NCI> AD/GAL−, AD/GAL+, $p < 0.01$.