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Noradrenergic sympathetic sprouting and cholinergic reinnervation maintains non-amyloidogenic processing of AβPP via M1 mAChRs

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Abstract

Alzheimer's disease (AD) is characterized by amyloid-beta (Aβ) plaques, hyperphosphorylated tau neurofibrillary tangles (NFTs) and cholinergic dysfunction. Cholinergic degeneration can be mimicked in rats by lesioning cholinergic neurons in medial septum. Hippocampal cholinergic denervation disrupts retrograde transport of nerve growth factor (NGF), leading to its accumulation, which subsequently triggers sprouting of noradrenergic sympathetic fibers from the superior cervical ganglia into hippocampus. Previously we reported that coincident with noradrenergic sprouting is the partial reinnervation of hippocampus with cholinergic fibers, and the maintenance of a M1 mAChR dependent long-term depression at CA3-CA1 synapses that is lost in the absence of sprouting. These findings suggest that sympathetic sprouting and the accompanying cholinergic reinnervation maintains M1 mAChR function. Interestingly, noradrenergic sympathetic and cholinergic sprouting have been demonstrated in AD postmortem human brain. Furthermore, M1 mAChRs have been a recent focus as a therapeutic target for AD given their role in cognition and non-amyloidogenic processing of amyloid beta-protein precursor (AβPP). Here we tested the hypothesis that noradrenergic sympathetic sprouting and the associated increase in cholinergic innervation maintains non-amyloidogenic AβPP processing that is dependent upon M1 mAChRs. Also, we investigated the effect of intrahippocampal $A\beta_{42}$ infusion on noradrenergic sympathetic and cholinergic sprouting. We found that $A\beta_{42}$ is not only toxic to central cholinergic fibers innervating hippocampus but prevents and reverses noradrenergic sympathetic sprouting and the accompanying cholinergic reinnervation. These findings reiterate the clinical implications of sprouting as an innate compensatory mechanism and emphasize the importance of M1 mAChRs as an AD therapeutic target.

Keywords

Alzheimer disease; amyloid beta; cholinergic fibers; muscarinic receptor M1; nerve growth factor; amyloid beta-protein precursor

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INTRODUCTION

Pathological hallmarks of AD include accumulation of amyloid-beta (Aβ) plaques and hyperphosphorylated tau neurofibrillary tangles (NFTs) along with degeneration of cholinergic innervation in cortex and hippocampus. These pathologies, along with soluble Aβ, blood brain barrier breakdown, oxidative stress, inflammation and gliosis interplay to induce accelerated neuronal degeneration and synaptic dysfunction [1].

Cholinergic dysfunction is the target of the primary treatment, acetylcholinesterase inhibitors, used in AD patients [2]. However, these drugs have limited benefit, likely due to decreased production of acetylcholine with disease progression. Recently, there is direct evidence that M1 muscarinic acetylcholine receptors (M1 mAChRs), which are the most abundant muscarinic receptor in cerebral cortex and hippocampus, play a critical role in cognition and AD pathogenesis [2–4]. Interestingly, activating M1 mAChRs elevates soluble amyloid precursor protein α (sAβPPα), and decreases Aβ plaques and NFTs [3, 5, 6]. On the contrary, pharmacological inhibition or genetic deletion of M1 mAChRs from 3xTgAD mice, which have cholinergic dysfunction, exacerbates cognitive impairments and increases the density of Aβ plaques and NFTs and the magnitude of gliosis [7, 8]. Thus, M1 mAChRs are a viable therapeutic target for AD and a better understanding of the beneficial effects of stimulating M1 mAChRs and preserving their function during cholinergic degeneration is critical [3].

The cholinergic dysfunction found in AD can be mimicked in rats by lesioning the cholinergic cell bodies of the medial septum (MS) [9–12]. Cholinergic denervation of hippocampus leads to nerve growth factor (NGF) accumulation due to loss of retrograde trafficking, and causes sprouting of noradrenergic sympathetic fibers. These fibers normally innervate the cerebral vasculature [13–18] and originate from the superior cervical ganglia (SCG) [16, 19]. We previously showed that coincident with the noradrenergic sympathetic sprouting is the reinnervation of dentate gyrus and area CA1 of hippocampus with cholinergic fibers [20]. Both of these fiber types are completely absent in rats with medial septal lesion combined with bilateral superior cervical ganglionectomy, suggesting that the cholinergic fibers, in addition to the noradrenergic fibers, originate from the SCG, although this remains to be determined [19, 20]. Importantly, we found that concurrent with sympathetic sprouting and cholinergic reinnevation is the maintenance of M1 mAChR dependent long term depression (mLTD) at hippocampal CA3-CA1 synapses [20]. Thus, we hypothesize that this sprouting is compensating for lost cholinergic function and is beneficial.

Importantly, noradrenergic sympathetic sprouting occurs in hippocampus of AD subjects [21]. Additionally, cholinergic sprouting has been demonstrated in close proximity to Aβ plaques in AD post-mortem human brain tissue, although whether this cholinergic sprouting is a consequence of noradrenergic sympathetic sprouting as in rats is not currently known [22, 23]. Given these findings from humans with AD, understanding the role of noradrenergic sympathetic sprouting and the associated cholinergic sprouting and how it interacts with Aβ is imperative and could have clinical implications. Thus, we wanted to investigate how endogenous AβPP processing is affected in rats with noradrenergic

sympathetic and cholinergic sprouting compared to rats with complete loss of cholinergic hippocampal innervation and no sprouting. We predicted that sprouting could sustain non-amyloidogenic processing of AβPP because it maintains M1 mAChR function [20]. Additionally, we wanted to determine if intrahippocampal treatment of $A\beta_{42}$ *in vivo* could induce or inhibit noradrenergic sympathetic and cholinergic sprouting. On one hand, Aβ leads to degeneration of cholinergic fibers in hippocampus which could increase NGF and stimulate sprouting. However, on the other hand, Aβ purified from AD patient brains has been shown to be toxic to SCG neuronal cultures [24]. Because of the documented benefits of sympathetic sprouting and cholinergic reinnervation on hippocampal synaptic function in rats with cholinergic degeneration, it is critical to determine whether Aβ accumulation will interfere with this potentially clinically relevant compensatory mechanism.

MATERIAL AND METHODS

All experiments were performed with Institutional Animal Care and Use Committee approval at the University of Alabama at Birmingham in accordance with NIH guidelines.

Animals

Adult male Sprague-Dawley rats, 2–5 months old (Charles River Laboratories) were used in all experiments. Animals were housed two per cage and were kept on a 12 hour light/dark cycle with ad libitum food and water. These studies include 4 animal groups: sham lesion with intact ganglia (control), MS lesion with intact ganglia (MS), MS lesion with ganglionectomy (MSGx) and sham lesion with ganglionectomy (Gx).

MS lesion and superior cervical ganglionectomy

Medial septal lesions and ganglionectomies were performed at 8–9 weeks of age. Rats were anesthetized using a ketamine (100 mg/kg) and xylazine (13 mg/kg) mixture administered intraperitoneally. Superior cervical ganglionectomies were performed as previously reported [10, 11, 20, 25–27]. After a neck incision, the SCG were visualized using a surgical microscope and bilaterally excised. Sham ganglionectomies involved the SCG being exposed but not removed. After SCG removal, medial septal lesions were performed. Cholinergic neurons in the medial septum were lesioned using either electrolytic stimulus (2 mA, 10 seconds) for immunohistochemistry experiments or using the immunotoxin 192-IgG-Saporin (Advanced Targeting Systems, San Diego, CA) which binds the low affinity pan neurotrophin receptor (p75NTR) for western blot experiments. 192-IgG-Saporin (0.5 μg toxin/ul PBS, total volume 2 uL) was injected stereotaxically over 5 minutes using a Hamilton syringe (AP +0.2, DV −5.8, L=0). Sham lesioned rats underwent the same procedure but received PBS only or electrode insertion with no current. Although rats with MS lesions induced either via electrolytic or saporin are used here, both lesion methods have been shown to have the same effect on M1 mAChR coupling [10, 25, 26].

Dicyclomine administration

Control and MS rats received intraperitoneal injections of M1 mAChR antagonist, dicyclomine (Sigma-Aldrich, St. Louis, MO, 8 mg/kg), each day for 4 weeks. Previously, this method was used in 3xTgAD mice and shown to increase Aβ pathology [28].

Cannulation and Aβ administration

Rats with MS lesion and intact ganglia were anesthetized via ketamine/xylazine (100 mg/13mg/kg, ip). Bilateral cannulas (Plastics One, Roanoke, VA) were implanted into region CA1 of hippocampus (AP-3.6 mm, DV-3.2mm, L ±2.1mm). Synthetic Aβ peptide (300 pM/day) was administered using an infusion pump (Alzet, Cupertino, CA) to control rats for 28 days. Two groups of MS rats received Aβ. One group received Aβ for 14 days beginning 16 days post-lesion after sprouting has been established. The second MS group received Aβ for 28 days starting 7 days post lesion prior to the onset of sprouting.

Western blot

Rats were anesthetized using 2.5% isoflurane and were decapitated and brain removed. The brain was partitioned such that the medial septum block was immediately stored in 4% PFA and acute hippocampal slices were prepared from the remaining block in artificial cerebral spinal fluid (aCSF) (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1 NaH_2PO_4, 26 $NaHCO_3$, and 11 glucose. Following this incubation, the CA1 and dentate gyrus regions were immediately dissected from the slices. The tissue was then homogenized in lysis buffer (T-PER Tissue Protein Extraction Reagent, ThermoScientific, Rockford, IL), including protease inhibitor. Samples containing 15 μg of protein were resolved on 4–20% precast gels (Invitrogen) and transferred to polyvinylidene difluoride membrane, blocked and incubated with anti-Aβ (1:750, Zymed) antibody overnight at 4°C. Blots were then incubated in HRP-conjugated secondary antibody (1:3000; Bio-Rad, Hercules, CA) for 2 hours at RT. Bands were detected using Western Lightning Plus-ECL chemiluminescence (Perkin Elmer, Waltham, MA). Densitometric quantification of immunopositive bands was performed with ImageJ and ImageQuant software. The ratio of α C-terminal fragment (αCTF, 8kDa) to β C-terminal fragment (βCTF, 10 kDa) to actin was determined and graphed.

Transcardial perfusion

Rats were anesthetized using 2.5% isoflurane and were transcardially perfused to remove blood from the brain with ice cold phosphate buffered saline (PBS) for 10 minutes followed by 4% paraformaldehyde (PFA) for 10 minutes. After perfusion, brain was removed and fixed in 4% PFA at 4°C for 48 hours.

Immunohistochemistry

Medial Septum—To confirm lesion completeness, serial sections (50μm in 6 series) of medial septum from all rats were prepared by vibratome and stored in antifreeze solution until staining. One series of sections (8 sections per rat) were washed and incubated in blocking buffer (0.3% Triton X-100, 10% normal donkey serum in PBS) for 90 minutes and then incubated in anti-choline acetyltranserase (ChAT) (1:300, EMD Millipore, Billerica, MA, AB144P) at 4°C overnight. The next day, sections were washed and then incubated in donkey anti-goat Alexa 488 (1:500, Invitrogen) for 90 minutes at RT followed by washing and a 10 minute application of DAPI. Sections were then mounted and coverslipped with Aquamount reagent and imaged on a fluorescent light microscope. A complete lesion was defined as 90% cholinergic cell loss from medial septum (Figure 1).

Hippocampus—One series of hippocampal sections (8 sections per rat) were washed and blocked in universal blocking buffer (UBB: 1% BSA, 0.2% non-fat powdered skim milk, 0.35% Triton X-100 in PBS) and then stored in anti-p75 neurotrophin receptor (p75-NTR) receptor [MC-192] (1:250, abcam, ab6172) and either anti-tyrosine hydroxylase (TH) (1:500, EMD Millipore, Billerica, MA, ab152) or anti-dopamine β hydroxylase (DβH) (1:500, abcam, Cambridge, MA, ab43868) at 4°C overnight. After washing, the sections were incubated in ImmPRESS anti-mouse Ig (1:10, Vector Labs, Burlingame, CA, MP-7402) and donkey-anti-rabbit Alexa 594 (1:500, Invitrogen, Grand Island, NY) in UBB for 90 minutes at RT. Next, the sections were washed and then stored in FITC Tyramide Plus in amplification buffer (1:1,500, Perkin Elmer) for 30 minutes at RT followed by washing and a 10 minute application of DAPI. Sections were then mounted and coverslipped with ProLong Gold antifade reagent (Invitrogen, Grand Island, NY) and imaged on an Olympus Fluoview 1000 laser scanning confocal microscope acquiring 30 stacks at 0.5 μm increments. For each rat, all 8 sections in the series were inspected for the presence of positive immunostaining in both regions CA1 and DG. At least 2 series were stained per rat and at least an N=4 rats were included in each data set. To verify that our anti-p75NTR staining was indeed working, we investigated the nucleus basalis region in the same sections and found the expected immunopositive cell bodies and processes (Supplemental 1d). Furthermore, p75NTR positive fibers could clearly be seen in sections stained together with those above from a control rat with no $A\beta_{42}$. (Supplemental 1a–c). To confirm the absence of nonspecific binding from secondary antibodies, we stained one series of sections in unison with the above experiment eliminating the addition of primary antibody (data not shown).

Statistics

All data are expressed as mean ± SEM. Statistical significance of optical density measured between groups by western blot was determined using one-way ANOVA, followed by Tukey post-hoc test using Origin software (OriginLab). The accepted level of significance was $p < 0.05$.

RESULTS

Sympathetic sprouting can be identified by the colocalization of antibodies to p75 neurotrophin receptor (p75NTR) and tyrosine hydroxylase (TH) in rats with a medial septal lesion

Previously, our lab and others have identified noradrenergic sympathetic sprouting induced by cholinergic denervation by morphological characterization using an antibody to tyrosine hydroxylase (TH), the rate limiting enzyme in the synthetic pathway for dopamine and norepinephrine. (TH) [14, 20, 27]. However, hippocampus is normally innervated by central noradrenergic fibers arising from the locus coeruleus, which are also positively labeled with the anti-TH antibody, preventing an unambiguous identification of peripheral versus central noradrenergic fibers. Therefore, to conclusively distinguish noradrenergic sympathetic sprouting from central noradrenergic fibers, we used a double staining strategy taking advantage of the selective expression of the pan neurotrophin receptor, p75NTR (green) by peripheral but not central noradrenergic fibers [29]. Thus, we performed double

immunofluoresence histochemistry using anti-p75NTR (green) and anti-TH (red) antibodies to identify peripheral versus central noradrenergic fibers. Using this approach, we find thick ribbon-like double labeled fibers in the hilar region of the dentate gyrus, separately identifying noradrenergic sympathetic axons (both green and red; yellow in merged image) from central noradrenergic axons (red only) (Figure 2d–f). Central cholinergic fibers and the cholinergic reinnervation that occurs as a consequence of noradrenergic sympathetic sprouting are also identified (green only) because central cholinergic neurons also express p75NTRs [29]. Double labeled fibers were absent in control (Figure 2a–c) and MSGx (Figure 2g–i) rats.

Non-amyloidogenic processing is maintained in MS rats with noradrenergic sympathetic sprouting and cholinergic reinnervation but not in MSGx rats where sprouting is absent

We have shown that sympathetic sprouting and the consequent increase in cholinergic innervation maintains M1 mAChR LTD [20], therefore we tested the hypothesis that sprouting should also maintain non-amyloidogenic AβPP processing, which requires M1 mAChRs. To determine this, we measured αCTF and βCTF protein levels in synaptosomal fractions from dissected CA1 and dentate gyrus subfields from all four animal groups: control (sham lesion + intact ganglia, N=7), Gx (sham lesion + ganglionectomy, N=7), MS (medial septal lesion + intact ganglia, N=7), and MSGx (medial septal lesion + ganglionectomy, N=7), and calculated the αCTF:βCTF ratio. The αCTF is generated by α-secretase cleavage of AβPP in the non-amyloidogenic pathway and the βCTF fragment is generated by β-secretase cleavage in the amylodiogenic pathway. Therefore, a higher αCTF:βCTF ratio indicates predominant non-amyloidogenic processing [30]. The αCTF:βCTF ratio is not different between control, Gx, and MS rats in area CA1 (Figure 3a, $p=1$) or DG (Figure 3b, $p=1$), indicating that non-amyloidogenic processing is maintained in MS rats that have sprouting. However, MSGx rats that do not have sprouting have a significant decrease in the αCTF:βCTF ratio in area CA1 ($p<0.001$) and DG ($p<0.005$) as compared to control, Gx and MS (Figure 3). These results emphasize the benefit of noradrenergic sympathetic and cholinergic sprouting in compensating for the lost cholinergic input to maintain non-amyloidogenic processing of AβPP.

M1 mAChRs maintain nonamyloidogenic processing in control and MS rats— M1 mAChRs are required for non-amyloidogenic AβPP processing [3, 5, 6]. To determine whether M1 mAChRs are involved in the maintained nonamyloidogenic processing, control (N=7) and MS (N=6) rats were treated *in vivo* with dicyclomine, the M1 mAChR antagonist previously shown to exacerbate Aβ pathology in 3xTgAD mice [28], and αCTF and βCTF protein levels were measured in synaptosomal fractions by Western blot. We found that dicyclomine decreases the αCTF:βCTF ratio in both the control ($p<0.001$ in CA1 and DG) and MS group ($p<0.001$ in CA1 and DG) (Figure 3a, b). This reconfirms our previous findings that M1 mAChRs are maintained in MS rats [20] that have noradrenergic sympathetic sprouting and the accompanying cholinergic sprouting and emphasizes the importance of M1 mAChRs on processing of endogenous AβPP.

Chronic Aβ is deleterious to central cholinergic fibers in hippocampus and does not induce sympathetic sprouting in control rats—Aβ is toxic to central

cholinergic neurons which should cause accumulation of NGF and stimulate noradrenergic sympathetic and cholinergic sprouting [15–17]. Therefore, we next wanted to determine if cholinergic degeneration caused by chronic intrahippocampal Aβ administration stimulates sprouting. Using double anti-p75NTR and anti-TH immunohistochemistry, we found a complete loss of fibers positive only for p75NTR, indicating a complete loss of cholinergic axons in area CA1 (Figure 4a–c) and DG (Figure 4d–f) in rats following 28 days of Aβ treatment. Furthermore, we found no fibers double labeled with anti-p75NTR and anti-TH, indicating the absence of noradrenergic sympathetic sprouting (Figure 4, N=5).

Chronic intrahippocampal infusion of synthetic Aβ limits noradrenergic sympathetic and cholinergic sprouting

The above data demonstrates that $A\beta_{42}$ does not induce sympathetic sprouting as a consequence of the cholinergic degeneration. Next to determine whether $A\beta_{42}$ interferes with the establishment of sprouting induced by electrolytic MS lesion synthetic $A\beta_{42}$ was delivered via cannula to area CA1 post-medial septum lesion at two different time points in separate groups of rats. In the first group, intrahippocampal $A\beta_{42}$ infusion began 7 days post-lesion and continued for 28 days (Figure 5, N=4). The goal of this experiment was to determine whether $A\beta_{42}$ administration at the time that noradrenergic sympathetic sprouting and cholinergic reinnervation are just beginning would prevent additional sprouting. In the second group, intrahippocampal $A\beta_{42}$ infusion began 16 days post-lesion and continued for 14 days (Figure 6, N=4). The goal of this was to determine whether the $A\beta_{42}$ administration after the establishment of sprouting would cause degeneration of the sprouted axons. Using double anti-p75NTR and anti-TH immunohistochemistry, we rarely observed noradrenergic sympathetic or cholinergic sprouting in rats that received intrahippocampal $A\beta_{42}$ infusion just as sprouting was beginning (Figure 5, 4/32 sections from 4 rats) or after the establishment of sprouting (Figure 6, 2/32 sections from 4 rats). Furthermore, in the few sections where sprouting was present, only 1 or 2 double labeled fibers were observed and the morphology was thin and delicate compared to the ribbon-like appearance that is more typical of sympathetic sprouting (Figure 2). These findings reveal that $A\beta_{42}$ is not only toxic to central cholinergic neurons, but also to peripheral sympathetic axons *in vivo*.

DISCUSSION

Here, we report the novel finding that noradrenergic sympathetic sprouting and the concomitant cholinergic sprouting maintain nonamyloidogenic processing of AβPP that is dependent upon M1 mAChRs. Also, we found that, even though reports show increased noradrenergic sympathetic sprouting in AD patients compared to age matched control subjects [21], chronic $A\beta_{42}$ infusion to hippocampus causes cholinergic axon degeneration but does not trigger noradrenergic sympathetic or cholinergic sprouting in control rats, suggesting that $A\beta_{42}$ accumulation is likely not the trigger for sprouting in humans. Furthermore, we found that $A\beta_{42}$ prevents and is deleterious to sprouting with only rare incidences of sprouting being identified. Aβ has been shown to be toxic to SCG neurons *in vitro* [24], but to the best of our knowledge, this is the first report to demonstrate that $A\beta_{42}$ is toxic to peripheral sympathetic axons *in vivo*. Previously, our lab has shown that noradrenergic sympathetic and cholinergic sprouting are able to maintain M1 mAChR

dependent mLTD [20]. Altogether, these findings suggest that noradrenergic sympathetic and cholinergic sprouting is compensatory; maintaining otherwise lost M1 mAChR function.

Considerable data including the present work supports that M1 mAChRs are a viable therapeutic target for AD [3, 20, 27, 31–35]. Due to this, selective M1 mAChR pharmaceuticals are being developed [36]. Interestingly, TBPB, a M1 mAChR allosteric potentiator, was reported to increase non-amyloidogenic processing of AβPP [36]. Also, the mechanism by which M1 mAChRs regulate AβPP processing is beginning to be understood. Specifically, M1 mAChRs and interacts with BACE1 to regulate its proteasomal degradation and regulate AβPP processing through a mitogen-activated protein kinase-dependent and protein kinase C (PKC)-dependent pathway [6, 37]. Additionally, disrupted coupling between M1 mAChRs and Gq proteins correlates with reductions of PKC activity and NMDA receptor density in post-mortem human brain from AD subjects [38]. While the relationship between M1 mAChRs and AβPP processing is now appreciated, the strongest recent evidence showing therapeutic potential of M1 mAChRs is that the removal of M1 mAChRs exacerbates all AD related phenotypes found in the 3xTgAD mice [8].

The finding that $A\beta_{42}$ is toxic to noradrenergic sympathetic and cholinergic sprouting is not surprising. Even though $A\beta_{42}$ is toxic to cholinergic fibers, which should increase NGF and trigger sprouting, Aβ can interact with p75NTRs to induce neuritic dystrophy [15, 39, 40]. In fact, application of Aβ isolated from AD patient brains has been shown to be toxic to superior cervical ganglia neurons, further supporting our findings [24]. Interestingly, noradrenergic sympathetic sprouting has been found predominately in Aβ plaque poor areas of post-mortem brain sections from AD subjects [41]. This raises the question of the timing of noradrenergic sympathetic sprouting in the AD disease process. Our data would suggest that chronic $A\beta_{42}$ both prevents and is deleterious to sprouting. Knowing that noradrenergic sympathetic sprouting is found in AD post-mortem human brain in regions that are Aβ plaque poor would suggest that this sprouting likely occurs early in the disease process [21, 41]. Taken together with our finding that noradrenergic sympathetic sprouting and the associated cholinergic sprouting maintain physiological function suggests that sprouting could be a novel therapeutic target improving M1 mAChR function for AD patients [20]. Importantly, NGF treatment, the trigger for noradrenergic sympathetic and cholinergic sprouting, is currently in clinical trials for AD patients and has shown benefit [42–44].

Here, we employed a cholinergic lesion rat model that recapitulates many of the hallmarks of AD [9, 10, 12, 45–49]. The intriguing aspect of this animal model is the ingrowth of noradrenergic sympathetic and cholinergic fibers into hippocampus, both of which have been identified in post-mortem AD hippocampus [11, 21, 22, 50]. We show that this sprouting maintains M1 mAChR function sustaining non-amyloidogenic processing of AβPP. Future studies should explore the benefit of noradrenergic sympathetic and cholinergic sprouting on other AD related deficits including hippocampal dependent learning. Altogether, our findings reiterate the potential for M1 mAChRs as a therapeutic target for AD and highlight the compensatory mechanisms of noradrenergic sympathetic and cholinergic sprouting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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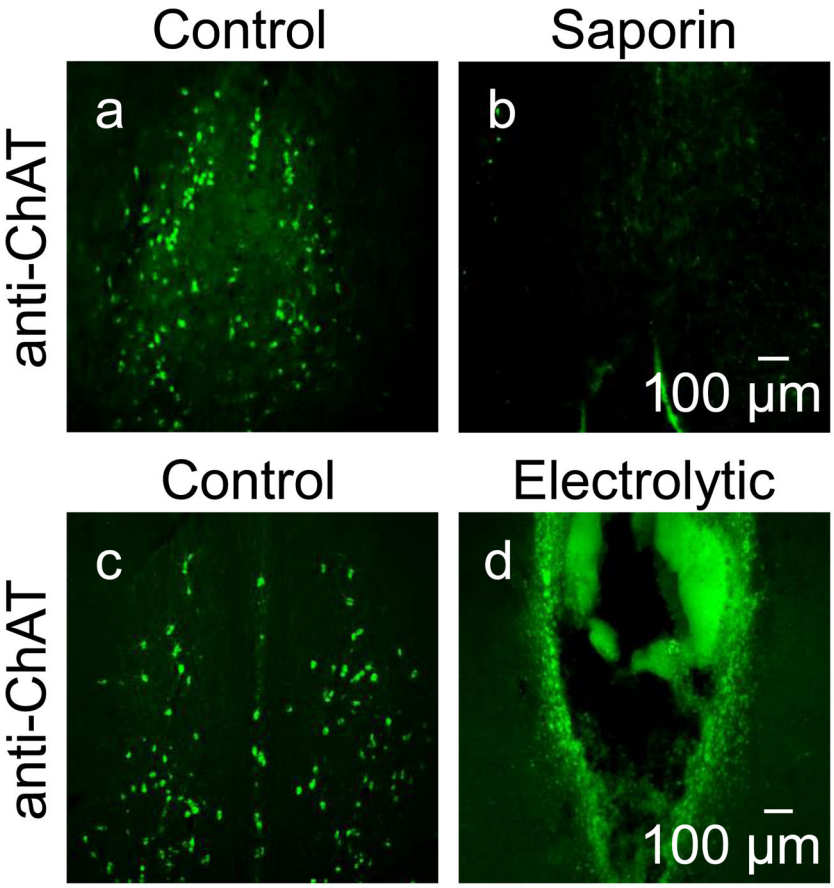

Figure 1.

MS lesions confirmed using immunohistochemistry with anti-ChAT (green) antibodies. In control rats (a, c) cell bodies of MS can be identified. However, with saporin (b) or electrolytic lesions (d), most of these cells are no longer present. A lesion was considered complete if less than 10% of cells were remaining.

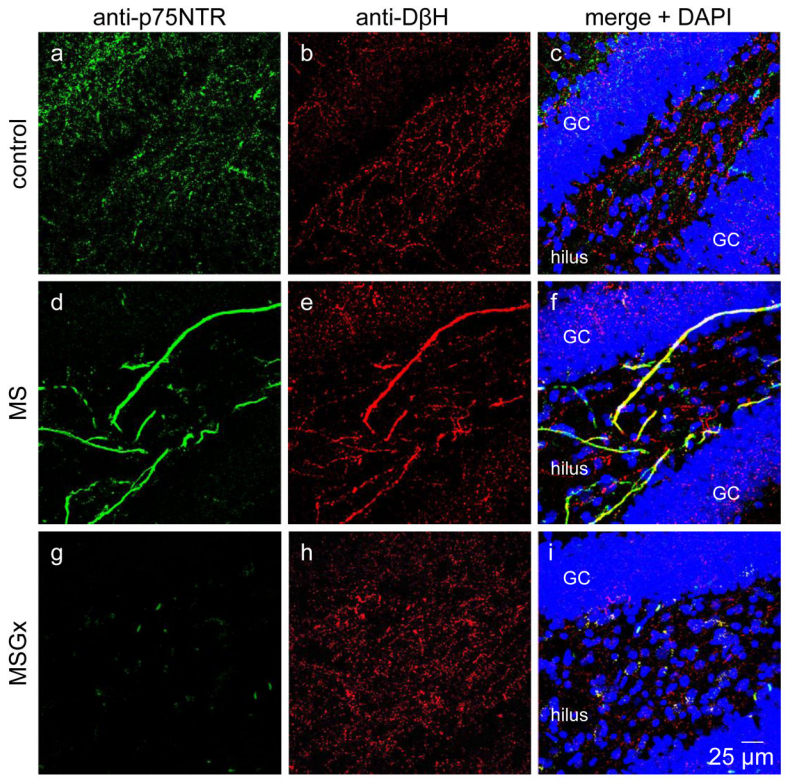

Figure 2.

Noradrenergic sympathetic sprouting can be identified using double immunohistochemistry by the colocalization (yellow) of anti-p75NTR (green) and anti-DβH (red) antibodies. Double labeling is absent in dentate gyrus from control (a–c) and MSGx (g–i) rats. Double labeled fibers are only present in MS rats (d–f). Confocal images obtained at 40x. GC, granule cell

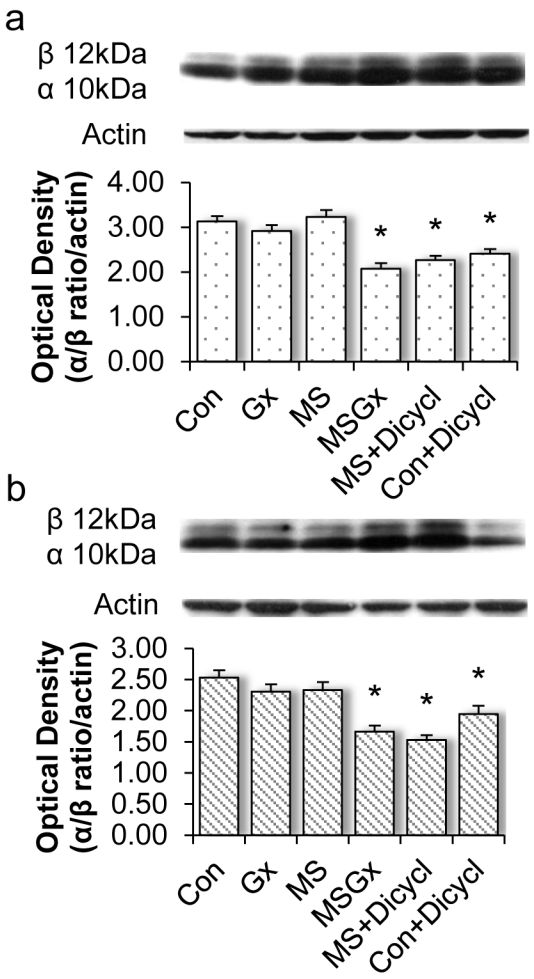

Figure 3.

M1 mAChR dependent non-amyloidogenic processing of AβPP is maintained in rats with noradrenergic sympathetic sprouting and cholinergic reinnervation. The αCTF/βCTF/actin ratio in area CA1 (a) and DG (b) was not different between control, GX or MS rats, demonstrating a compensatory role of sprouting on non-amyloidogenic AβPP metabolism. Control and MS rats treated systemically with the M1 mAChR antagonist dicyclomine had a significant decrease in the αCTF/βCTF/actin ratio, indicating that the maintenance of non-amyloidogenic AβPP processing is dependent upon M1 mAChRs. pyr, pyramidal cell; s. rad., stratum radiatum; s.l.m., stratum lacunosom moleculare; GC, granule cell

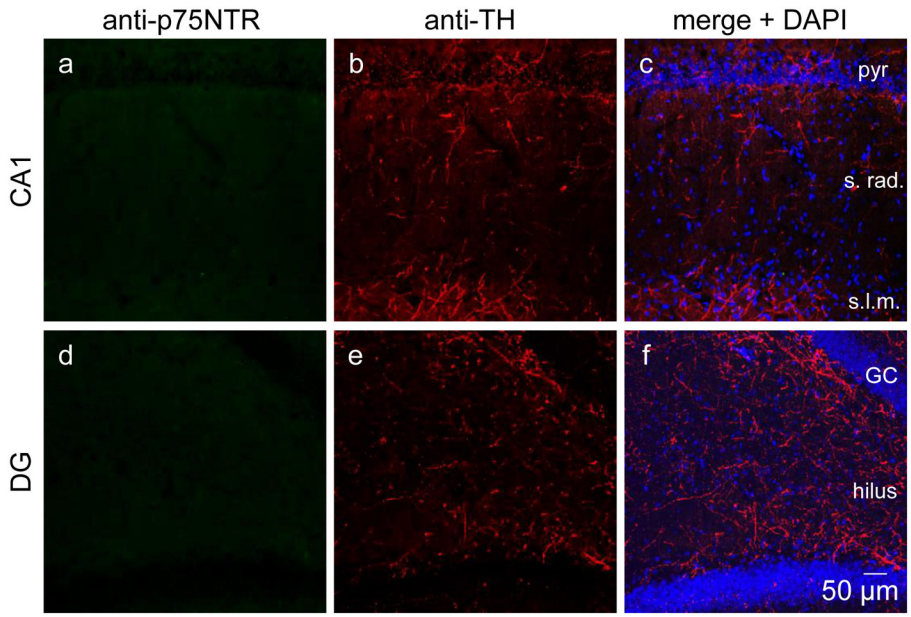

Figure 4.

$A\beta_{42}$ is toxic to central cholinergic fibers in control rats. Intrahippocampal infusion of synthetic $A\beta_{42}$ for 28 days into hippocampus is detrimental to all central cholinergic axons in hippocampus. Using p75NTR immunohistochemistry, no cholinergic fibers can be identified in area CA1 (a–c) or DG (d–f) (N=5). Confocal images obtained at 20x. pyr, pyramidal cell; s. rad., stratum radiatum; s.l.m., stratum lacunosom moleculare; GC, granule cell

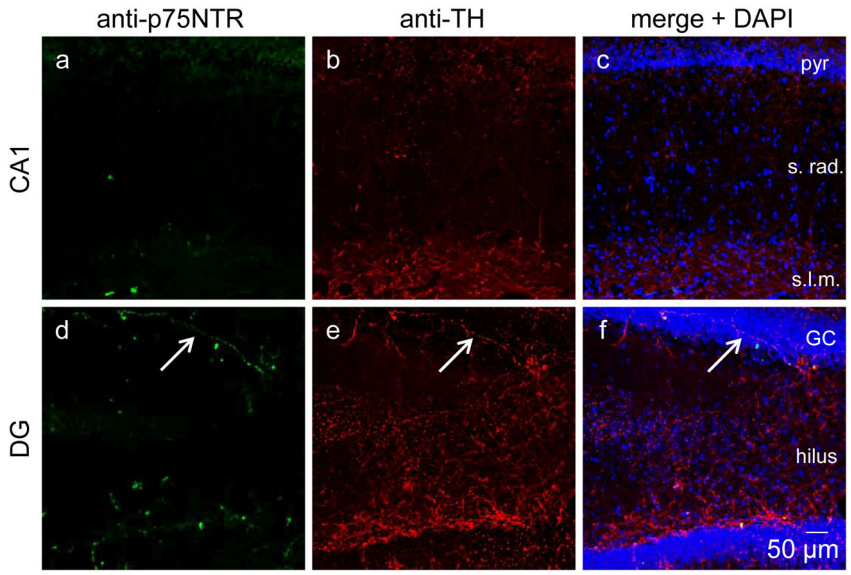

Figure 5.

$A\beta_{42}$ prevents noradrenergic sympathetic and cholinergic sprouting in MS rats. Intrahippocampal infusion of synthetic $A\beta_{42}$ began 7 days post-lesion and continued for 28 days. Noradrenergic sympathetic or cholinergic sprouting could not be identified in area CA1 (a–c). Rarely, noradrenergic sympathetic sprouting could be identified in DG (d–f, arrow) however, the abundance is drastically reduced compared to sprouting seen without $A\beta_{42}$ (Figure 2d–f) (N=4). Confocal images obtained at 20x. pyr, pyramidal cell; s. rad., stratum radiatum; s.l.m., stratum lacunosom moleculare; GC, granule cell

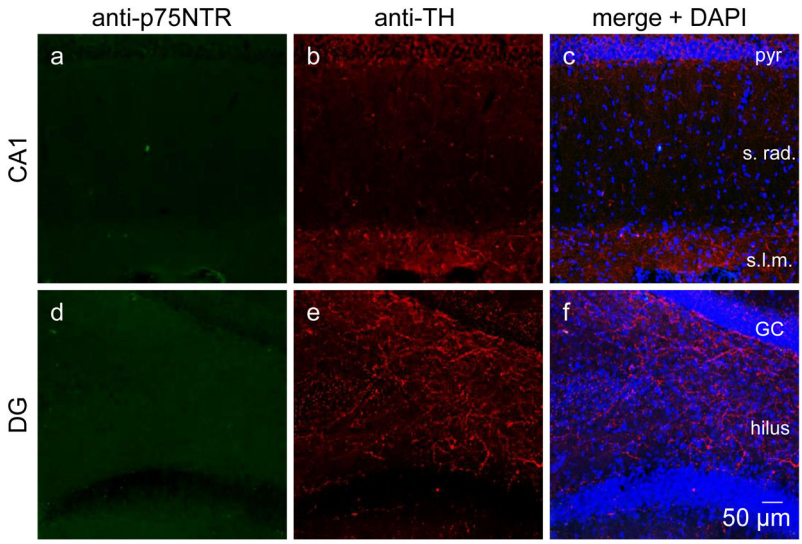

Figure 6.

$A\beta_{42}$ interferes with noradrenergic sympathetic and cholinergic sprouting in MS rats. Intrahippocampal infusion of synthetic $A\beta_{42}$ began 16 days post-lesion and continued for 14 days. Noradrenergic sympathetic or cholinergic sprouting could not be identified in area CA1 (a–c) or DG (d–f) (N=4). Confocal images obtained at 20x. pyr, pyramidal cell; s. rad., stratum radiatum; s.l.m., stratum lacunosom moleculare; GC, granule cell