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Partial depletion of striatal dopamine enhances penetrance of cognitive deficits in a transgenic mouse model of Alzheimer's disease

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Abstract

Parkinson's disease (PD) and Alzheimer's disease (AD) are recognized to coexist on a spectrum of neurodegeneration, and it has been proposed that molecular interactions among pathogenic proteins are a basis for the overlap between these two diseases. We instead hypothesize that degeneration of the nigro-striatal dopaminergic system enhances the clinical penetrance of early stage AD. To determine the effect of striatal dopamine on the pathological effects in an experimental model of AD, APP_{SWE}/PS1 E9 mice received striatal injections of the neurotoxin 6-hydroxydopamine (6OHDA). Animals were tested in a Barnes maze protocol and a water T-maze protocol at different ages to determine the onset of cognitive impairment. APP_{SWE}/PS1 E9 mice that received 6OHDA injections showed significant impairment in Barnes maze performance at an earlier age than controls. Additionally, at 12 months of age APP_{SWE}/PS1 E9+6OHDA mice demonstrated worse behavioral flexibility in a task-switch phase of the water T-maze than other groups. To determine the neuroprotective effects of dopaminergic neurotransmission against A β ₄₂ toxicity, neuronal branch order and dendrite length was quantified in primary medium spiny neuron (MSN) cultures pretreated with increasing doses of the D₁ and D₂ receptor agonists before being exposed to oligomerized A β ₄₂. While there was no difference in A β peptide levels or plaque burden between groups, in murine MSN culture dopaminergic agonists prevented a toxic response to A β ₄₂. Depletion of dopamine in the striatum exacerbated the cognitive impairment seen in a mouse model of early stage AD, which may be due to a protective effect of dopaminergic innervation against A β striatal neurotoxicity.

Keywords

6OHDA; amyloid; cognitive impairment; Parkinson's disease; Alzheimer's disease

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Introduction

Dementia is a major, increasing challenge to older individuals, public health, and health care systems (“CDC - Healthy Brain Initiative,” n.d.). Dementia in older adults is a complex convergent trait that derives most commonly from an idiosyncratic mix of Alzheimer's disease (AD), Lewy body disease (LBD), and vascular brain injury (Hyman et al., 2012; Montine et al., 2014). Although effective interventions for vascular brain injury exist, there is yet no disease modifying therapy for AD or LBD. Indeed, interventions to prevent, stop, or slow the progression of these neurodegenerative diseases would relieve untold suffering and be a very valuable contribution to the sustainability of health care systems.

AD is a chronic illness with a preclinical stage characterized by progressive decline in cognitive function and brain regional accumulation of senile plaques and neurofibrillary tangles, that culminates in severe cognitive and behavioral changes that are diagnosed as dementia (Sperling et al., 2011). LBD comprises Parkinson's disease (PD) and Dementia with Lewy body disease (DLB); both are characterized by regional accumulation of Lewy bodies, marked degeneration of the nigrostriatal dopaminergic system, and dendritic simplification, but not loss, of striatal medium spiny neurons (MSNs) (Zaja-Milatovic et al., 2006, 2005). MSN dendritic simplification has been proposed to be secondary to loss of dopaminergic innervation of MSN dendritic spine shafts resulting in unopposed glutamatergic stimulation of dendritic spine heads (Obeso et al., 2000). Although DLB includes dementia by definition, patients with PD also commonly develop dementia, and the clinical distinction between PD with dementia (PDD) and DLB is based arbitrarily on the interval between onset of motor vs. cognitive symptoms (Aarsland and Kurz, 2010; Feldman et al., 2014a, 2014b; Marder et al., 1995). There is extensive pathological overlap between PDD and DLB, and both commonly co-exist with AD pathologic changes (Montine et al., 2012). Indeed, several groups have reported that coincident pathologic changes of AD and LBD occur more commonly than would be predicted by chance alone, raising the possibility that somehow the apparently disparate disease processes act synergistically (Dugger et al., 2012; Gomperts et al., 2008; Irwin et al., 2012). These findings have led others to test hypotheses concerning shared risk factors or common pathogenic mechanisms for AD and LBD, especially molecular interactions between amyloid β ($A\beta$) peptides, tau species, and α -synuclein, the critical protein constituents of senile plaques, neurofibrillary tangles, and Lewy bodies, respectively (Clinton et al., 2010; Guo et al., 2013).

Another possibility is that AD and LBD are molecularly distinct, but LBD enhances the penetrance of latent or prodromal AD such that individuals with AD and LBD develop AD-related dementia with AD neuropathology that would be insufficient to cause dementia in the absence of LBD. In support of this hypothesis, we observed that patients with PDD have a CSF AD biomarker profile expected for preclinical AD rather than that of AD dementia (Montine et al., 2010); results that have been broadly replicated by others (Alves et al., 2014, 2010; Siderowf et al., 2010). Moreover, we recently demonstrated that the amount of $A\beta_{42}$ and paired helical filament (PHF)-tau in brain of patients who died with dementia is lower in those with co-existent LBD (Postupna et al., 2015). Although there are several possible interpretations of these now broadly validated observational data from CSF and autopsy brain of patients with LBD, all are consistent with the hypothesis that LBD

enhances the clinical penetrance of AD, making it more likely for someone with both diseases to cross the diagnostic threshold for dementia at lower burden of AD. Here, we have tested this hypothesis experimentally.

Materials and Methods

Drugs

6-hydroxydopamine hydrobromide (6OHDA) (Sigma, St. Louis, MO) was dissolved in sterile PBS at 2.5µg/µL. SKF81297 and quinpirole (Fisher Scientific, Pittsburgh, PA) were dissolved in sterile water for a stock concentration of 1mM.

Animals

APP_{swe}/PS1 E9 mice were kept on a 12hr light/dark cycle in an SPF vivarium according to University of Washington IACUC protocols. They were injected with 1µL 6OHDA or vehicle bilaterally into the dorsal striatum (+0.5mm, ±1.5mm, -3.0mm from Bregma) at 2.5-3 months of age. All tests are in accordance with University of Washington protocols and Washington state law.

Behavioral Analysis

Barnes maze—To assess hippocampal-dependent spatial and working memory, we used a modified Barnes maze protocol (Yang et al., 2013). The Barnes maze apparatus (San Diego Instruments, San Diego, CA) is a disc 1.0 m in diameter raised 75 cm off the floor containing 18 possible escape holes, one of which leads to a dark escape box. Bright light and fan noise were used to increase motivation for escape. Animals were trained to escape the maze into the hidden box by allowing them to explore the maze for 60 seconds and then placing them in the box with a food pellet prior to any testing. Animals were then trained over a 3 day acquisition phase to learn the location of the escape box within the maze using spatial cues in the testing room (4 trials the first day, then 3 trials per day with a 2 minute inter-trial interval). Trials ended when animals located the escape box or 5 mins had elapsed. If animals did not find the escape box within the time limit they were shown the box and gently encouraged to enter it. In order to increase the cognitive load on the animals and engage working memory, after day 3 the escape box was moved to a different randomized location and animals were again given 3 trials to learn the new location (2 minute inter-trial interval) in a reversal learning scenario. Latency to escape, distance traveled, and errors made (investigations into decoy escape holes) were measured. This protocol was performed on mice at 3, 6, and 9 months of age. In order to determine memory retention in these mice, at 12 months animals were tested in a 4 day protocol without the change in box location, then 10 days later were tested in the protocol described above.

Water maze—The water T-maze is a spatial memory test that is independent of motor ability (Darvas and Palmiter, 2011). The apparatus consists of two distinct arms 14 inches in length, one black and one white, which branch off of a longer stem (18 inches) and curve backwards to prevent animals from seeing the end of the arm before entering it. There is an escape platform at the end of one arm at all times. Animals were placed in the stem and allowed to swim to the platform. The trial ended when the animal located the platform or 5

mins had elapsed. Animals were given 10 trials per day with 3-5 mins rest between trials. On days 1-3 the escape platform was always in the same color arm (black or white), and the maze was flipped at random between trials. On days 4-8, the escape platform was always on the left or the right side, and the maze was again flipped at random. Color and side assignments were randomized between cohorts. Latency to escape, number of “correct” (entering the escape arm first) trials per day, and entries into the incorrect arm were measured.

Rotarod—To measure motor deficits, at 9 months of age mice were trained on the rotarod (San Diego Instruments, San Diego, CA). Constant speeds of 12 rpm, 16 rpm, and 20 rpm with 5 trials for each speed were used for 5 consecutive days. On the 6th day mice were challenged with 5 trials of rpms increasing from 5-30. The maximum time for each trial was 180s.

Immunostaining

Medium spiny neurons were cultured from the lateral ganglionic eminence (LGE) of wild type (WT) embryonic day 14 pups. Cells were incubated in DMEM/F12 supplemented with 10% B27 neuronal supplement and 5% penicillin/streptomycin for 17 days. On day 17 cells were pretreated with indicated concentration of the D₁R agonist SKF81297 or the D₂R agonist quinpirole for 20 min and then incubated with 10 μ M A β ₁₋₄₂ (Bachem Americas INC, Torrance, CA) for 24 hrs (Li et al., 2013). Slides were stained with mouse monoclonal anti-MAP2 antibody (Abcam, Cambridge, MA), rabbit monoclonal anti-DARPP32 (Cell Signaling, Danvers, MA), and Alexafluor488 or 555 (Invitrogen, Carlsbad, CA). Cells were analyzed by an investigator blind to cell treatment. On average, three cells per slide (two slides per treatment group) were selected at random for analysis in three separate experiments. Dendrite length and branching were analyzed using Neurolucida software (MBF Bioscience, Williston, VT).

Luminex quantification of A β

Cellular lysates were generated from dissected cortex, hippocampus, and striatum as previously described (Yang et al., 2013). In brief, tissues were homogenized by pulse sonication (10 seconds) in 10ul buffer (20 mmol/L Tris-HCl, pH 7.5; 150 mmol/L NaCl; 1 mmol/L phenylmethylsulfonyl fluoride; protease inhibitor cocktail) per mg of tissue (wet weight), followed by centrifugation at 30,000 $\times$ g for 30 minutes. Supernatants (so-called soluble fraction) were collected and stored at -80C prior to assay. The remaining pellets were further solubilized in 5 mol/L guanidine-HCl buffer (same volume to weight ratio), clarified by centrifugation (30,000 $\times$ g for 30 minutes), and the supernatants (so-called insoluble fraction) collected and frozen (-80°C). Human A β ₄₀ and A β ₄₂ Luminex kits (Invitrogen, Grand Island, NY) were used for quantitative analysis of amyloid from soluble and insoluble fractions according to the manufacturer's protocol.

Catecholamine measurements

7 days after behavioral testing was completed, animals were sacrificed by rapid decapitation and the brains were removed. The left hemisphere was rapidly sectioned into 2mm slices and a 1mm punch was taken from the dorsal striatum at the level of the 6OHDA lesion.

Tissue punches were flash-frozen immediately and stored at -80°C. HPLC coupled with electrochemical detection (Coulochem III electrochemical detector, Thermo Fisher Scientific, MA) was used to measure DA. All raw measurements of DA were normalized to total protein content.

Histology

The 2 mm slices from the left hemisphere described above were immersion fixed in 4% paraformaldehyde overnight. After fixation, slices were embedded coronally in paraffin blocks and cut serially on a microtome (Leica, Buffalo Grove, IL) into 5 µm thick sections. Every twentieth section (100 µm interval) was stained for H&E to ensure the lesion was in the correct anatomical location and to select consistent brain sections for all groups to perform Aβ immunohistochemistry. An anterior section at the level of the anterior commissure was sampled from each mouse as well as a posterior section at the level of the posterior hippocampus. WT pilot samples were stained with rabbit polyclonal anti-TH antibody at 1:1000. For amyloid staining, all tissue sections were first boiled in citrate buffer for antigen retrieval (Yang et al., 2013) and then immunostained simultaneously with mouse monoclonal 6E10 antibody (1:5000, Covance, Seattle, WA) to ensure equal and ubiquitous staining of amyloid plaques. Slides were then imaged at 20× magnification on a Nikon fluorescent microscope (Melville, NY) and at the same exposure to perform quantitative image analysis. Each image was uploaded to ImageJ (NIH, Bethesda, MD) where both anterior and posterior sections of cortex, hippocampus, and striatum underwent uniform thresholding to quantify plaque burden. Plaque burden was quantified as the total area of all measured plaque in a region over the total area of the region. Each anterior and posterior region was averaged per mouse to give a region specific plaque burden estimate. All plaque data was within previously established percentiles for APP_{swe}/PS1 E9 mice (Cimino et al., 2013; Garcia-Alloza et al., 2006).

Statistics

Statistical analyses were performed using GraphPad Prism (San Diego, CA). α was set to 0.05. *P* values without symbols are results from one- or two-way ANOVA. Correction for repeat pair comparisons following one- or two-way ANOVA were performed using the method of Bonferroni; symbols represent corrected *P* values, *e.g.*, *0.05, *0.01, **0.001, or ***0.0001. Other symbols for corrected *P* values follow the same format when comparing multiple groups, and are described in text.

Results

First, we determined the location and estimated the extent of 6OHDA-mediated damage to striatal dopaminergic terminals in a pilot experiment that used WT mice (*n* = 6). Mice were injected with 6OHDA or vehicle at 2 months of age, sacrificed 2 weeks later, and their brains processed for TH immunohistochemistry. Coronal sections of cerebral hemispheres from this pilot group revealed loss of dopaminergic innervation confined to the dorsal striatum while leaving the ventral striatum intact (Fig 1A). The remainder of our *in vivo* experiments utilized WT and APP_{swe}/PS1 E9 mice that received bilateral striatal injections of 6OHDA or vehicle at 2 months of age and were then tested for behavioral deficits at

different time points. We confirmed the durability of the lesions by testing motor function on the rotarod apparatus at 9 months of age. As expected from our pilot experiment, both WT ($*P<0.05$) and APP_{swe}/PS1 E9 ($***P<0.001$) mice treated with 6OHDA showed significant deficits in an accelerating rotarod test (Supplemental Figure 1). Tissue punches through the dorsal striatum of these mice that corresponded to the site of TH depletion observed in our pilot experiment (Fig 1A, dashed circles) confirmed that 6OHDA-exposed WT ($**P<0.01$) and APP_{swe}/PS1 E9 ($*P<0.05$) mice had significantly and comparably reduced DA levels 10 months after the lesion was introduced compared to untreated and vehicle treated controls (Fig 1B).

WT and APP_{swe}/PS1 E9 mice with and without 6OHDA treatment were tested in a modified Barnes maze protocol at 3, 6, 9, and 12 months of age to assess hippocampal-dependent spatial and working memory as reflected in latency to escape, errors, and distance traveled. There were no significant differences among groups at 3 or 6 months of age (Supplemental figures 2 and 3); however, by 9 months the APP_{swe}/PS1 E9+6OHDA mice performed significantly worse than animals in the other groups (Fig 2). Latency to escape was increased for each training day and APP_{swe}/PS1 E9+6OHDA mice were particularly deficient in cognitive flexibility, as evidenced by a reduced ability to determine the new location of the escape box on the challenge day (Fig 2A). Indeed, two-way ANOVA comparing all groups ($P<0.0001$), all trials ($P<0.0001$), and their interaction (non-significant) in Figure 2A had significantly increased latency to escape for APP_{swe}/PS1 E9+6OHDA mice compared to APP_{swe}/PS1 E9+Veh ($****P<0.0001$) for trial 11, APP_{swe}/PS1 E9+6OHDA mice vs. WT+6OHDA ($+++P<0.001$) for trial 11, and APP_{swe}/PS1 E9+6OHDA mice vs. WT+Veh mice ($^^P<0.001$) for trial 11. Errors (Fig 2B) were significantly different on Day 4 at 9 months of age in APP_{swe}/PS1 E9+6OHDA mice compared with APP_{swe}/PS1 E9+Veh ($*P<0.05$) and WT+Veh mice ($**P<0.01$). Distance traveled (Fig 2C) also was significantly increased on Day 4 at 9 months of age in APP_{swe}/PS1 E9+6OHDA mice compared with WT+6OHDA mice ($***P<0.001$).

By 12 months of age, vehicle-treated APP_{swe}/PS1 E9 mice had further deteriorated and were no longer significantly different from APP_{swe}/PS1 E9+6OHDA mice in latency to escape (Fig 3A). This indicates that APP_{swe}/PS1 E9+6OHDA have an earlier onset of impairment in higher cognitive functions, such as cognitive flexibility, rather than a more severe impairment in overall cognitive function. Surprisingly, neither APP_{swe}/PS1 E9 group was significantly different from WT groups at this age. It is possible that the repeated testing in this apparatus confounds the data at this late stage, as mice may have learned to amend their search strategies over time.

To address the confound of animal learning and to ensure that behavioral impairments in the Barnes maze were not due to motor deficits, we further investigated the cognitive ability of all mice by testing the mice in a water T-maze apparatus at 12 months of age. The use of water as a motivator is also stronger than the Barnes maze apparatus, and thus is more sensitive to cognitive impairments. Motor impairment does not influence this test as the primary outcome measure is number of correct trials per day without regard to speed. Animals were trained to find an escape platform using arm color (black or white) as the cue, and then were challenged by switching the cue to arm direction (left or right). All groups

learned the initial task equally well (Fig 4A; two-way ANOVA for the four groups vs. day had $P < 0.0001$ for day but was not significant for group or interaction). Following the task switch (Fig 4B), two-way ANOVA had $P < 0.0001$ for day, $P < 0.001$ for group, and non-significant interaction, with corrected post tests significant for Days 2 ($***P < 0.001$) and 3 ($**P < 0.01$) and APP_{swe}/PS1 E9+6OHDA mice performing poorest on each day except the last when all groups were equivalent. On Day 3 following the task switch, APP_{swe}/PS1 E9+6OHDA mice were significantly worse at learning the task switch than all other groups ($**P < 0.01$ for each corrected pair comparison) (Fig 4B). These data indicate that while APP_{swe}/PS1 E9+6OHDA mice are not impaired in initial learning in this task compared with controls, they are significantly impaired in cognitive flexibility, as evidenced by their impaired ability to adjust to the new task.

To determine whether the exacerbated cognitive deficit seen in 6OHDA treated APP_{swe}/PS1 E9 mice corresponded to an increased A β burden, we measured A β plaque load and A β tissue concentration in these mice after sacrifice at 12 months of age. A β plaque load was measured by thresholding analysis in the cerebral cortex, hippocampus, and striatum of all mice and plotted as a fraction of total area (Supplemental Figure 4A). As expected, A β plaques were not detected in WT mice. There was no significant difference in plaque area between APP_{swe}/PS1 E9+6OHDA and vehicle groups. Tissue concentrations of A β peptides were determined by LUMINEX on flash-frozen tissue from cerebral cortex, hippocampus, and striatum. As with the A β plaque burden, there were no significant differences among groups in soluble or insoluble A β_{40} or A β_{42} in any brain region (Supplemental Figure 4B). These results indicate that the impairment seen in the water T-maze in 12 month old APP_{swe}/PS1 E9+6OHDA mice is not a result of increased A β accumulation as soluble or insoluble peptides, or in plaques.

Our behavioral results show an earlier onset of impairment in 6OHDA-exposed APP_{swe}/PS1 E9 mice that was ultimately matched by older vehicle-exposed APP_{swe}/PS1 E9 mice. In the absence of differences in A β levels between these groups, we hypothesized that reduced striatal dopamine might make MSNs more vulnerable to a given amount of A β and thus increase the penetrance of A β toxicity. We tested this hypothesis by determining whether DA receptor agonists protected cultured MSNs from A β_{42} -induced neurite damage. MSNs from E14 WT pups were pretreated with increasing doses of the D₁-like receptor agonist SKF81297 or the D₂-like receptor agonist quinpirole before being exposed to 10 μ M A β_{42} for 24 hours (Fig 5). A β_{42} treatment alone reduced MSN total neurite length (Fig 5B) to about one half ($**P < 0.01$) and higher branch order (Fig 5C) to about one-third ($***P < 0.001$) of vehicle controls, while neurite length and branch order were not affected by D₁R and D₂R agonist treatment alone. However, neurite length (Fig 5B) varied significantly across all exposed groups ($P < 0.001$), with 0.5 and 1.0 μ M SKF81297 pre-treatment having a modest protective effect against the neurotoxic effects of A β_{42} ($*P < 0.05$). Lower concentration (0.5 μ M) quinpirole pre-treatment did not protect against A β_{42} -induced reduction in neurite length ($**P < 0.01$) but neurite length in MSNs treated with 1.0 μ M quinpirole followed by A β_{42} was not significantly different than vehicle + A β_{42} control. Analysis of neurite branch order yielded similar results (Fig 5C); partial protection from the neurotoxic effects of A β_{42} by SKF81297 pre-treatment and near complete

protection at the higher concentration of quinpirole. Results from pre-treatment with 5.0 μ M SKF81297 or quinpirole were not significantly different than 1.0 μ M of either drug (not shown). These data demonstrate that dopamine-like agonists, especially the ligand of D₂-like receptors, protected against A β ₄₂ toxicity to MSNs in culture.

Discussion

Neuropathologic studies have repeatedly recognized an association between the pathologic changes of AD and LBD; however, the mechanism(s) underlying this association are not known. Motivated by results from observational studies of PD patients in whom we measured CSF biomarkers of AD and brain autopsy studies in which we quantified A β ₄₂ (Alves et al., 2014, 2010; Montine et al., 2010; Postupna et al., 2015; Siderowf et al., 2010), we tested the hypothesis that a partial lesion to the nigrostriatal dopaminergic system would enhance the penetrance of cognitive deficits in a transgenic mouse model of AD. Our experimental results broadly support this hypothesis, and suggest one potential mechanism may be a loss of dopamine-mediated protection from A β ₄₂ neurotoxicity in the striatum.

Dorsal striatal injection of 6OHDA at 2 months of age sufficient to produce a local reduction in tissue dopamine concentration and modest impairment of performance on the rotarod shifted significant impairment in a modified Barnes maze protocol to earlier onset in APP_{swe}/PS1 E9 mice compared to the same mice exposed to vehicle. Although APP_{swe}/PS1 E9+6OHDA mice initially were able to learn the location of an escape hole, overnight retention was impaired compared to control groups with more severe deficits apparent in a test of cognitive flexibility by changing the location of the escape hole. Likewise, all mice were able to learn to find an escape platform in the water maze using the initial cue-directed escape, but APP_{swe}/PS1 E9+6OHDA mice were much slower to learn a change in the rules and to find the escape platform using a new cue-based directional rule. Together these data indicate that the APP_{swe}/PS1 E9+6OHDA mice have impaired cognitive flexibility with earlier onset rather than a specific learning deficit. This cognitive deficit has been observed in more severe models of both AD and dopamine depletion; indeed, AD transgenic mice at older ages, but not as young as 9 months of age, and mice with near complete ablation of striatal dopaminergic terminals, have been shown to have impaired cognitive flexibility (Barr et al., 2007; Darvas and Palmiter, 2011). Our results indicate that combining the two stressors led to earlier expression of significant cognitive impairment of this type.

As in people with AD dementia, we confirmed that A β peptide accumulation was not only present in the cerebral cortex and hippocampus but also in the striatum of APP_{swe}/PS1 E9 (Dugger et al., 2012; Klunk et al., 2007). Indeed, although not widely appreciated as a site of AD pathologic change or A β peptide accumulation, neuroimaging data suggest that A β fibril accumulation as detected by PET tracers occurs first in the striatum, and subsequently is surpassed by cerebral cortex and hippocampus as AD progresses to more advanced stages (Klunk et al., 2007). Moreover, striatal A β accumulation has been included in the new consensus guidelines for the neuropathologic evaluation of AD, since striatal A β is often associated with transition from preclinical to prodromal disease (Hyman et al., 2012). As in brain from patients with AD dementia, A β plaque load in this mouse model was lower in striatum than in cerebral cortex (Hyman et al., 2012). The relative tissue concentrations of

insoluble A β peptides in caudate vs. cerebral cortex of APP_{swe}/PS1 E9 mouse brains were consistent with data from brains of individuals with AD, which vary from higher in the striatum to equivalent in the two regions to lower in striatum than cerebral cortex depending on stage of AD, the form of A β peptide, and region of cerebral cortex (Woltjer et al., 2009). In our experiments overall, in fact, tissue levels of A β peptides were *not* altered by our partial 6OHDA lesion of the nigrostriatal dopaminergic system. While experimental design did not allow for A β measurements to be taken at 9 months, given the persistent impairment in cognitive flexibility seen in the water T-maze in the older mice, it seems unlikely that differences in A β would be the mechanism for the accelerated cognitive impairment observed at 9 months. For these reasons, we determined the effects of dopamine receptor agonists on protection against A β ₄₂ toxicity in MSNs. Pretreatment with dopamine receptor agonists protected against A β ₄₂-induced neurotoxicity to cultured MSNs, a result that is similar to previous studies using murine hippocampal neuronal cultures (Jürgensen et al., 2011). Interestingly, the D₂ receptor agonist quinpirole was more effective at protecting MSNs against A β ₄₂ insult than the D₁ receptor agonist, and at the higher concentration afforded near complete neuroprotection. This result was somewhat unexpected, as D₂-like receptors are generally considered to be autoreceptors, and previous studies have shown a potent effect of D₁ receptor agonists (Jürgensen et al., 2011, p. 1). Although exogenously applied receptor-specific agonists are not comparable to endogenous neurotransmitter release from neurons, these data hint at a mechanism by which healthy dopamine neurons may protect their targets from A β insult. We also recognize that MSN in cell culture are neither in appropriate proportion to others striatal cells nor make connections like neurons in brain, but nevertheless our results suggest a potential role for indirect pathway striatal projection neurons, generally expressing D₂ receptors, in protection against neurotoxic effects of A β peptides.

Our results show that partial dopaminergic degeneration in the dorsal striatum is associated with earlier expression of cognitive impairment in a transgenic model of AD, and offer one explanation for the apparent synergistic association between AD and PD, for brain autopsy results that suggest an interaction between these two diseases, and for the CSF biomarker profile observed in patients with PD dementia (Alves et al., 2014, 2010; Montine et al., 2010; Postupna et al., 2015; Siderowf et al., 2010). We chose double transgenic APP_{swe}/PS1 E9 mice for these experiments in order to determine whether a partial lesion of the nigrostriatal dopamine system would accelerate onset of cognitive impairment in experimental AD, the equivalent of increased clinical penetrance. Intoxication with 6OHDA models PD in so far as it causes degeneration of dopaminergic neurons. We chose 6OHDA intoxication over transgenic models of PD that produce dopaminergic degeneration primarily to control the amount and location of dopaminergic damage, and to remove the possibility of other molecular interactions between products of APP_{swe}/PS1 E9 and transgenes in PD models, *e.g.*, interaction between A β peptides and mutant α -synuclein. 6OHDA intoxication also permits generalization of our results to other conditions that damage nigrostriatal dopamine neurons, especially environmental toxicants. Indeed, since the vast majority of older adults have A β accumulation in brain to some degree, our results provide a possible explanation for how known environmental toxicants to the dopaminergic system could promote cognitive decline with advancing age. Alternatively, our data may

help explain why behaviors such as exercise and social activity, both known to increase dopaminergic activity, may be protective against AD dementia despite no apparent effect on cerebral amyloid burden (Nation et al., 2011). Finally, our results provide some support to the possibility that D₂R agonists might delay clinical presentation, or perhaps even act as neuroprotectants, in individuals with striatal A β accumulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) TH staining showing 6OHDA lesion site 2 weeks after injection in a WT animal. Dashed circles indicate tissue punch location for HPLC analysis. (B) Dopamine quantification by HPLC analysis of tissue punches taken from the dorsal striatum. Symbols in Figure are for results from 1-way ANOVA with Bonferroni's multiple comparison test for data from WT or APP_{swe}/PS1 E9 (AD) mice (** $P < 0.01$ or * $P < 0.05$). $n = 5-10$

Figure 2.

Barnes maze behavioral analysis at 9 months. (A) Latency to escape into escape box is measured over trials and days. Box was relocated on day 4 (new location). Two-way ANOVA was significant for all groups ($p < 0.0001$) and all trials ($p < 0.0001$) without significant interaction. Corrected repeat pair comparisons had $****P < 0.0001$ for $APP_{swe}/PS1$ E9+6OHDA mice compared to $APP_{swe}/PS1$ E9+Veh, $+++P < 0.001$ for $APP_{swe}/PS1$ E9+6OHDA mice vs. WT+6OHDA mice, and $^^P < 0.001$ for $APP_{swe}/PS1$ E9+6OHDA mice vs. WT+Veh mice. (B) Investigation into decoy escape holes (errors) presented as the mean of all trials per day were significantly different on Day 4 ($P < 0.01$) with corrected pair comparisons showing significant increase in $APP_{swe}/PS1$ E9+6OHDA mice compared with $APP_{swe}/PS1$ E9+Veh ($*P < 0.05$) and WT+Veh mice ($**P < 0.01$). (C) Total distance traveled presented as the mean of all trials per day also was significantly on Day 4 at 9 months of age ($P < 0.001$) with corrected pair comparisons showing significant increase in $APP_{swe}/PS1$ E9+6OHDA mice compared with WT+6OHDA mice ($***P < 0.001$). $n=12-13$

Figure 3.

Barnes maze behavioral analysis at 12 months. (A) Latency to escape into escape box is measured over trials and days i) prior to and ii) after a 1 week interval to determine retention. (B) Investigation into decoy escape holes (errors) presented as the mean of all trials per day i) prior to and ii) after a 1 week interval. (C) Total distance traveled presented as the mean of all trials per day i) prior to and ii) after a 1 week interval. $n=11-12$

Figure 4.

Water maze behavioral analysis at 12 months. (A) Initial cue-directed escape learning is presented as total correct trials (out of 10) per day. All groups learned the initial task equally well (two-way ANOVA for the four groups vs. day had $P < 0.0001$ for day but $P > 0.05$ for group or interaction). The maze is flipped at random between trials and the escape platform is always on the same colored side. (B) Strategy-shift acquisition is measured by side-directed escape after initial cue-directed escape is learned. Two-way ANOVA had $P < 0.0001$ for group, $P < 0.0001$ day, and non-significant interaction with corrected post tests significant for Days 2 ($*P < 0.001$) and 3 ($**P < 0.01$) and APP_{swe}/PS1 E9+6OHDA mice performing poorest on each day except the last when all groups were equivalent. On Day 3 following the rule switch, corrected repeat pair comparisons showed that APP_{swe}/PS1 E9+6OHDA mice were significantly impaired at learning the rule switch than all other groups ($^{++}P < 0.01$ for each). $n=10-12$

Figure 5.

Medium spiny neurons (MSNs) cultured from WT E14 LGE treated with Aβ₄₂. (A) MSNs pretreated with indicated dosage of the D₁ agonist SKF81297 (top) or the D₂ agonist quinpirole (bottom) and then treated with either vehicle or 10 μM Aβ₄₂ for 24 hours, stained with rabbit MAP2. (B) Total neurite length ($P < 0.001$) and (C) % neurites with branch order 3 ($P < 0.0001$) for MSNs varied significantly across treatment groups. Aβ₄₂ for 24 hr significantly reduced MSN neurite length ($**P < 0.01$) and branch order ($***P < 0.001$). Pretreated with SKF81297 showed partial protection of MSNs from Aβ₄₂ toxicity ($*P < 0.05$, $**P < 0.01$), while pretreatment the higher concentration of quinpirole yielded neurite length and branch order that was not significantly different compared to vehicle treated controls.