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Catecholaminergic Neuronal Loss in Locus Coeruleus of Aged Female Dtg APP/PS1 Mice

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Abstract

Alzheimer's disease (AD) is the most common type of dementia afflicting the elderly. In addition to the presence of cortical senile plaques and neurofibrillary tangles, AD is characterized at autopsy by extensive degeneration of brainstem locus coeruleus (LC) neurons that provide noradrenergic innervation to cortical neuropil, together with relative stability of dopaminergic neuron number in substantia nigra (SN) and ventral tegmental area (VTA). The present study used design-based stereological methods to assess catecholaminergic neuronal loss in brains of double transgenic female mice that co-express two human mutations associated with familial AD, amyloid precursor protein (APP_{swe}) and presenilin-1 ($PS1_{\Delta E9}$). Mice were analyzed at two age groups, 3–6 mos and 16–23 mos, when deposition of AD-type β-amyloid (Aβ) plaques occurs in cortical brain regions. Blocks of brain tissue containing the noradrenergic LC nucleus and two nuclei of dopaminergic neurons, the SN and VTA, were sectioned and sampled in a systematic-random manner and immunostained for tyrosine hydroxylase (TH), a specific marker for catecholaminergic neurons. Using the optical fractionator method we found a 24% reduction in the total number of TH-positive neurons in LC with no changes in SN-VTA of aged dtg APP/PS1 mice compared with non-transgenic controls. No significant differences were observed in numbers of TH-positive neurons in LC or SN-VTA in brains of young female dtg APP/PS1 mice compared to their age-matched controls. The findings of selective neurodegeneration of LC neurons in the brains of aged female dtg APP/PS1 mice mimic the neuropathology in the brains of AD patients at autopsy. These findings support the use of murine models of Aβ deposition to develop novel strategies for the therapeutic management of patients afflicted with AD.

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Keywords

Substantia Nigra; Ventral Tegmental Area; Unbiased Stereology; APP/PS1 Mice; Tyrosine Hydroxylase

Alzheimer's disease (AD) is a debilitating, age-related neurological disease characterized by progressive deterioration of cognitive function, including severe impairments in memory and learning; deposition of congophilic β-amyloid (Aβ) plaques and neurofibrillary tangles containing ubiquinated *tau* protein; and, neuronal loss (Alzheimer 1907; Aletrino et al., 1992; Mirra et al., 1993; Busch et al., 1997). The pathological markers for the diagnosis of AD, the deposition of amyloid plaques and tangles, appear first in hippocampus and surrounding temporal lobe of the brain, and in later stages spread to all the cortical areas (Hyman et al., 1984; Braak and Braak, 1991; West et al., 1994). AD appears to selectively affect neuronal systems associated with cognitive and sensory processes such as hippocampus and cortex (Vogels et al., 1990; Busch et al., 1997; Aletrino et al., 1997), while sparing motor systems and their underlying biological substrate. At autopsy the brains of AD patients have significant reductions in total numbers of locus coeruleus (LC) neurons, the major source of brain norepinephrine (NE) (Swanson and Hartman, 1976; Busch et al., 1997), compared to the relative stability of LC neurons in normal aging (Mouton, et al., 1994; Ohm et al., 1997). In contrast, the substantia nigra (SN) and ventral tegmental area (VTA), two mesencephalic nuclei that project to striatum and neocortex, respectively, are relatively spared in concert with stable motor function in AD.

The introduction of transgenic strategies for the *in-vivo* expression of genetic mutations associated with familial AD, including the amyloid precursor protein (APP) and presenilin-1 (PS1), have provided important tools for understanding neural reactions to the deposition of mutant Aβ proteins in the mouse brain and developing novel approaches for the therapeutic management of AD in humans (Games et al., 1995; Hsiao et al., 1995; Malherbe et al., 1996; Hardy, 1997; Johnson-Wood et al., 1997; Strurchler-Pierrat et al., 1997; Morgan et al., 2000; Wang et al. 2003). In line with the view of gender differences reported in AD (Molsa et al., 1982; Jorm et al., 1987; Hagnell et al., 1992; Letenneur et al., 1994; Brayne et al., 1995; Fratiglioni et al., 1997, 2000), female dtg APP/PS1 mice appear to accumulate Aβ at an earlier age and to deposit more amyloid plaques in the hippocampus than the age-matched males (Wang et al. 2003; Callahan et al., 2001). One of these mouse lines co-express the so-called Swedish APP mutation (APP_{swe}) and the E9 presenilin-1 ($PS1_{\Delta E9}$) mutations (Borchelt et al., 1997). By 3–4 months of age these mice express high levels of mutant APP, PS1 and Aβ, and by 5 months of age deposit substantial numbers of Aβ–containing amyloid plaques which, like other lines of single and double transgenic mice, closely resemble the histological appearance of those found in AD (Frautschy et al. 1998; Holcomb et al., 1998; McGowan et al., 1999; Gordon et al., 2000; Selko, 2001). To help characterize the neuropathological similarity between AD and dtg APP/PS1 mice, we used unbiased stereological approaches to quantify total neuron numbers in the noradrenergic LC and dopaminergic SN-VTA in two groups of young and aged dtg APP/PS1 female mice and age-matched wild-type littermate controls.

Materials and Methods

Mice

Brains from 27 female mice were raised at the Laboratory of Experimental Gerontology at the Gerontology Research Center (GRC, NIA/NIH) in Baltimore, MD. The numbers per group, ages, and genotypes of the mice were as follows: 3–6 mos old dtg APP/PS1 [(APPswe, PS1dE9)85Dbo/J; PrP promoter] (n=6, young dtg APP/PS1); and 16–23 months old (n=7, aged dtg APP/PS1); and, age-matched, non-transgenic littermate controls (n=5 young, n=9 aged).

Mice were group housed (2–5) in plastic cages with corncob bedding, with *ad lib.* access to food (NIH formula 07) and filtered water in a vivarium maintained on a 12:12 h light:dark cycle, and a temperature of 22 +/− 2°C. The colony was certified as specific pathogen free for the following pathogens: mouse pneumonia virus, sendai virus, hepatitis virus, reovirus, lymphocytic choriomeningitis, Theiler Martin encephalomyelitis virus, ectromelia (poxvirus), minute virus of mouse and mocplasma pulmonis. The GRC is accredited and animal care and treatment followed the guidelines of the American Association for the Accreditation of Laboratory Animal Care.

Tissue Preparation

Mice were sacrificed by perfusion with 4% paraformaldehyde, and brains were removed and stored at −80°C until sectioning. Each brain was serially sectioned in the coronal plane on a cryostat at an instrument setting of 50μm. For stereological studies the LC and SN-VTA were sampled in a systemic-random manner, as detailed elsewhere (Mouton 2002).

Immunohistochemistry

For visualization of TH-positive neurons, systematic-random sections through the LC, SN and VTA, a total 8–10 sections per region per brain, were collected in 12-well plates and washed in 0.1 M PBS, incubated in 1% hydrogen peroxide for 30 minutes at room temperature (RT), washed again in PBS 0.1 M, and placed in 0.3% Triton X-100 for ten minutes RT. Sections were washed in PBS 0.1 M then transferred into 5% normal goat serum in 0.1 M PBS for 30 minutes at RT to block non-specific binding. Sections were incubated overnight in rabbit anti-tyrosine hydroxylase antibody (polyclonal, Chemicon International, Temecula, CA, USA) diluted to 1:3000 with 2% normal goat serum and 0.3% Triton X-100 in PBS at 4°C. After incubation, sections were washed in PBS 0.1 M and incubated in biotinylated secondary anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA) with normal goat serum in PBS 0.1 M for 90 minutes at RT. Sections were washed in PBS 0.1 M and re-incubated for another 90 minutes in ABC solution from the Vectorstain Kit (Vector Laboratories, Burlingame, CA, USA) at RT. Sections were rinsed in PBS 0.1M and colorized using DAB (10 mg DAB, 40 ml PBS 0.1M) for six to ten minutes. Additional sections from each group were immunostained for qualitative assessment of TH-immunopositive fibers in hippocampus, and cortex. All TH-immunostained sections were lightly counterstained in a 0.1% solution of cresyl violet, rinsed, dehydrated through an ascending graded series of alcohol, and cleared in xylene and coverslipped with DPX. To

visualize amyloid deposits, an adjacent set of sections through each brain was counterstained with Congo red, as reported previously (Lee et al., 2005).

Stereology

Total numbers of TH-positive in two reference spaces (LC and SN-VTA) were quantified using the optical fractionator method (West et al., 1991) as previously detailed (Long et al., 1998; Mouton et al., 2002; Lei et al., 2003; for review of relevant stereological techniques, see Mouton 2002). For both reference spaces, the sampling fractions were as follows: section sampling fraction for LC (ssf=1) and for SN/VTA (ssf=0.333), the number of sections sampled divided by the total number of sections; the area sampling fraction for LC (asf=0.2844) and for SN/VTA (asf=0.4444), the area of the sampling frame divided by the area of the x-y sampling step; and, the thickness sampling fraction for LC (tsf=0.5882) and for SN/VTA (tsf=0.625), the height of the disector divided by the section thickness. The reference space on each section was outlined under low power magnification (4X NA=0.10), and TH-positive cells were visualized under oil immersion magnification (60X, NA=1.4). The counting item was the presence of TH immunoreactivity in a cell with a neuronal phenotype. To avoid artifacts (e.g., lost caps) at the sectioning surface, a guard volume of 2–3 um was used. Sampling was continued to 100 to 150 neurons per reference space until a mean coefficient of error (CE) for each group was between 5 and 10% (Gundersen et al. 1999). The average section thickness for both regions was 16 ± 0.27 (SEM) μm.

Statistical Analysis

Differences between groups were assessed using the Student's two-tailed t-test, with statistical significance at the $p<0.05$ level.

Results

To expand our initial qualitative examination of TH-positive neurons in dtg APP/PS1 and control mice (Figure 1), total numbers of TH immunopositive neurons in LC, SN-VTA of female dtg APP/PS1 mice and non-tg littermate controls were quantified by design-based stereology. In the group of older dtg APP/PS1 mice and age-matched, non-transgenic littermate controls there were significantly fewer TH-positive neurons (24%) in the LC ($p < 0.01$; Figure 2). In a follow-up study in young mice there was no genotype effect on the total number of TH-positive neurons in the LC (Figure 3). For both young and old mice there were no differences in the total numbers of TH-positive neurons in SN-VTA in dtg APP/PS1 compared to age-matched controls (Figure 4). Qualitative assessment of sections through the hippocampus, and cortex indicated a robust loss of TH-positive fiber staining in these regions (data not shown).

Discussion

The underlying neurobiological basis for the severe, progressive cognitive decline and underlying neuropathological changes in AD remains unknown. To date, studies to characterize the neuropathological effects in transgenic mouse models of AD have focused on neurodegeneration in hippocampus (Calhoun et al., 1998; Casa et al., 2004; Schmitz et

al., 2004; Dickson 2004), the brain region thought to contain the critical memory circuits that underlie short-term memory in the mammalian brain. The LC provides tonic noradrenergic innervation to hippocampus and other cortical brain regions, and is thought to play an important role in the regulation of normal cognitive abilities such as attention, learning and memory (German et al. 2005, Aston-Jones et al., 1999). During normal aging these cognitive functions remain generally stable (Kawas et al., 2000) in concert with stability in the total number of TH-positive neurons in LC (Mouton et al., 1994; Ohm et al. 1997). In contrast, cognitive abilities diminish in AD in association with degeneration of transmitter-specific subcortical nuclei, including noradrenergic (Busch et al., 1997; Swaab et al., 1997), cholinergic (Vogels et al., 1990) and serotinergic neurons (Aletrino et al., 1992). Importantly, the catecholaminergic neuron loss in AD is selective for TH-positive neurons in LC that synthesize and release noradrenaline, while dopaminergic SN and VTA neurons are generally spared in concert with the extrapyramidal motor abilities mediated by these nuclei (Lyness et al., 2003).

The initial purpose of this study was to test whether expression of human APP and PS1 mutations was associated with loss of TH positive neurons in LC. To test this idea, we applied design-based stereology to a group of dtg APP/PS1 female mice aged 16–23 months and their age- and gender-matched, non-tg littermate controls. The results of these studies showed a significant 24% reduction of TH-positive neurons in the LC of dtg APP/PS1 mice compared to their controls, which confirmed our hypothesis. Careful immunostaining in the cortical projection regions from the LC revealed a loss of TH fibers in the hippocampus and cortex. To test whether this genotype effect was selective for the LC, we used the same stereology approaches to quantify the total number of TH-positive neurons in the SN-VTA region. This study revealed no differences in SN-VTA, supporting the view that the observed loss of TH-positive neurons in the aged female dtg APP/PS1 mice was limited to the LC.

In a follow-up study we addressed the question of whether the loss of LC neurons in the dtg APP/PS1 mice represents a degenerative vs. developmental disorder, i.e., an effect that is present in older but not younger mice, rather than an effect present at birth. To test this idea we used design-based stereology to quantify numbers of TH-positive neurons in a group of young dtg APP/PS1 mice and non-tg controls. The lack of a genotype effect in these young mice indicates that the observed differences reflect a degenerative, rather than development effect.

The finding of selective catecholaminergic neurodegeneration bears an apparent similarity to autopsy findings in AD patients compared to age-matched controls (Manaye et al., 1995; Busch et al., 1997; Swaab et al., 1997). It would be premature, however, to speculate that this apparent similarity arises from the same mechanism in AD patients and dtg APP/PS1 mice. Among the possible mechanisms are that the observed LC degeneration stems from retrograde degeneration of cortical projections arising in the LC. In this scenario an initial degeneration in the terminal projections in cortical tissue undergoing reactive inflammation to Aβ peptides could lead to the eventual degeneration of TH-positive neurons in the LC. Alternatively, loss of noradrenergic innervation from the pontine brainstem, secondary to primary loss of TH neurons in LC, could be an early event in the pathogenic cascade that includes neuroglia proliferation and activation and the formation of the characteristic

amyloid plaques in AD. Further studies in the dtg APP/PS1 mice are needed to carefully characterize the temporal pattern of LC degeneration in association with the appearance of Aβ deposits. In one recent study, Heneka et al. (2006) used chronic treatment with N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP-4) to mice that express a single APP mutation to induce LC degeneration. Interestingly, though these mice normally show relatively light deposition of Aβ peptides in cortical tissue, after chronic DSP-4 treatment there was substantially greater deposition of Aβ plaques, robust neurogliosis in cortical regions, and reduced spatial memory (Heneka et al., 2006). Thus, loss of noradrenergic innervation to cortical tissue appeared to stimulate deposition of insoluble Aβ peptides, possibly in association with activation of adrenergic receptors on neuroglial cells.

Previous studies have reported AD-type pathology in single (APP) and double (APP/PS1) transgenic mouse models of AD (Guela et al., 1998, Guenette and Tanzi, 1999, Callahan et al., 2001, Reilly et al., 2003), including deficits in cognitive performance (Richard et al., 2003, Huang et al., 2003, Palop et al., 2003), increased neuroglial activation (Bradt et al., 1998, Schwab et al. 1997, Meda et al., 1995) and degeneration of neurons in hippocampal formation but not in LC (Casas et al., 2004; Schmitz et al., 2004; for review, see German and Eisch 2004). Here we present evidence that aged female dtg APP/PS1 mice show an AD-type loss of noradrenergic neurons in LC. Among the possible explanations for this difference is variability in the individual APP and PS1 mutations; differences in the resultant deposition of Aβ; and differences in the ages of mice examined across these studies. Our future research will continue to examine possible mechanisms that underlie this degeneration in APP/PS1 mice. Understanding these mechanisms could help to better appreciate the biological basis of age-related neurogeneration, and possibly lead to novel strategies for the therapeutic management of AD in the elderly population.

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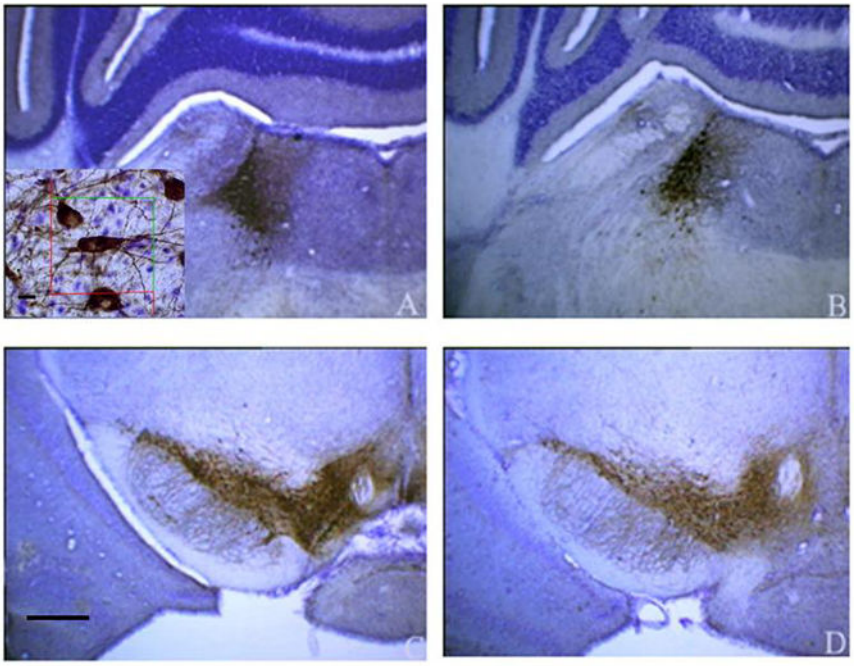

Figure 1.

Low magnification (4X) photomicrographs of TH-positive neurons in (A) LC from non-tg littermate controls aged 3–6 mos; (B) LC from dtg APP/PS1 mice aged 16–23 mos; (C) SN-VTA from non-tg littermate controls aged 3–6 mos; (D) SN-VTA from dtg APP/PS1 mice aged 16–23 mos. Insert: High magnification (60 x) with an optical disector of TH positive cells. Scale bar= 50μm

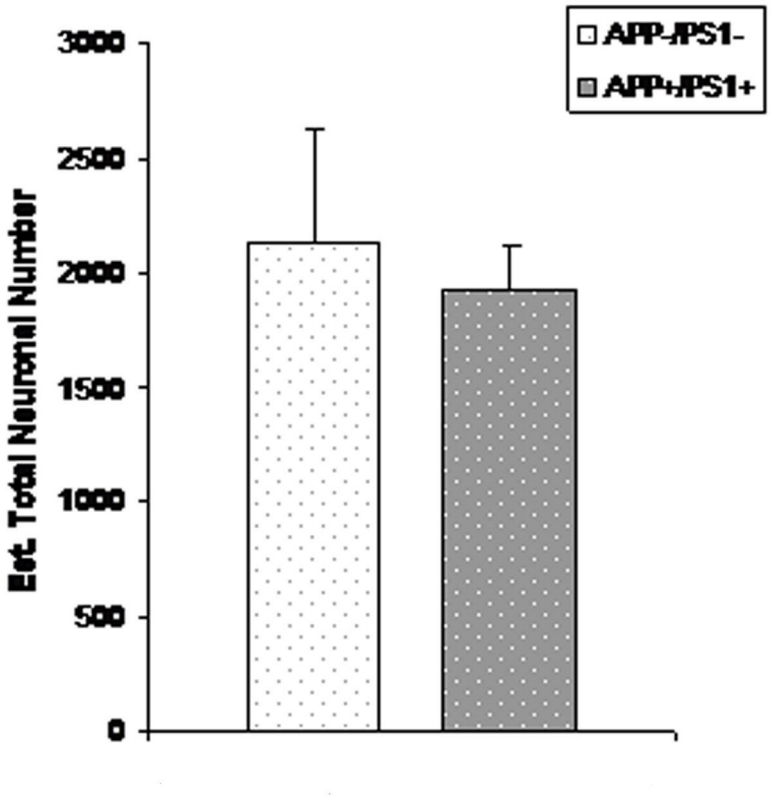

Figure 2.

Mean (SEM) total number of TH-positive neurons in LC of 16–23 month old female APP/PS1 mice (n=7) and age-matched non-tg littermate controls. (n=9); $p<0.01$.

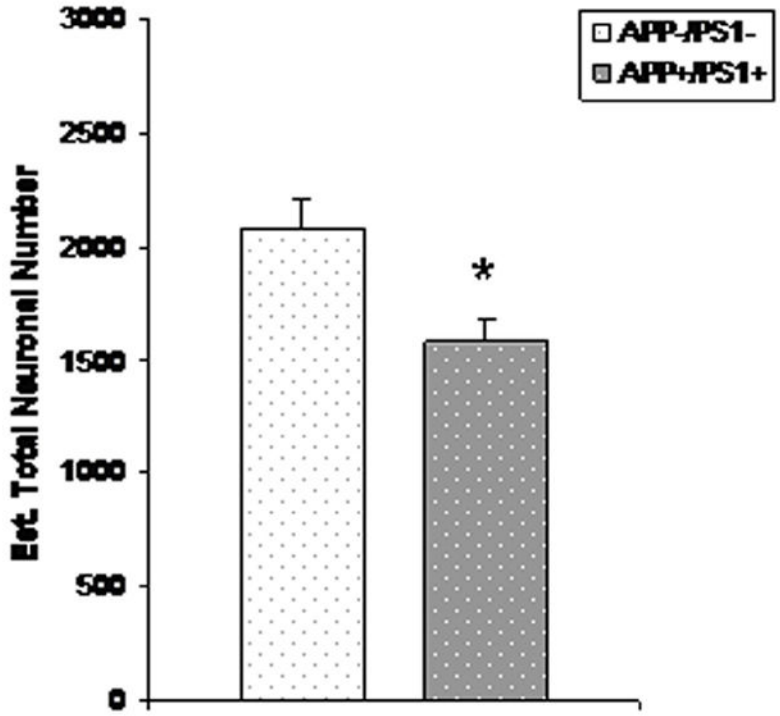

Figure 3.

Mean (SEM) total number of TH-positive neurons in LC of 3–6 month old female APP/PS1 mice (n=7) and age-matched non-tg littermate controls (n=9).

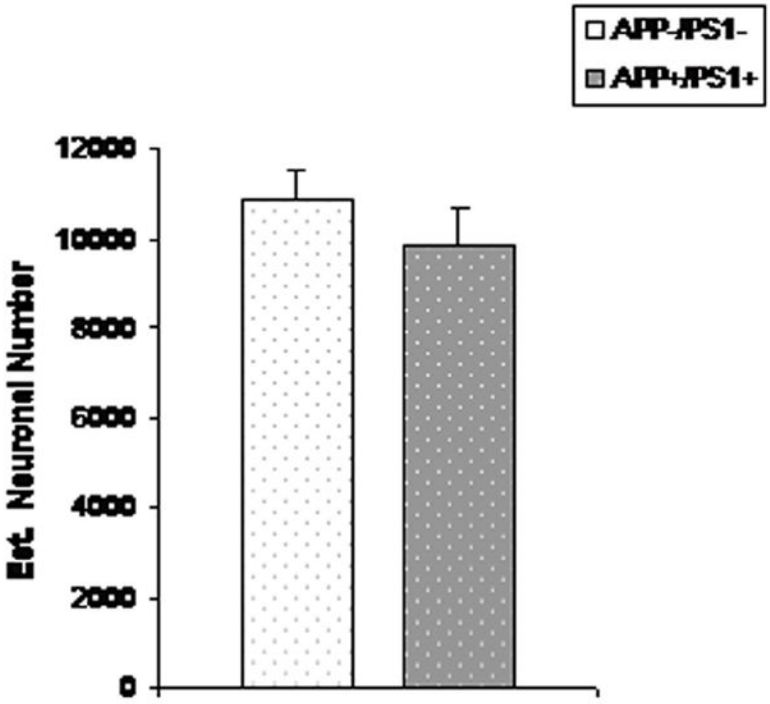

Figure 4.

Mean (SEM) total number of TH-positive neurons in SN-VTA of 16–23 month old female APP/PS1 mice (n=7) and age-matched non-tg littermate controls. (n=9).