Evidence of oxidative stress in young and aged DJ-1-deficient mice

Arjun V. Raman [b,c,1], Vivian P. Chou [a,b,1], Jennifer Atienza-Duyanen [b], Donato A. Di Monte [a,b,d], Frederick P. Bellinger [c], Amy B. Manning-Boğ [a,b,*]

[a] SRI International, Center for Health Sciences, Menlo Park, CA, USA
[b] The Parkinson's Institute, Department of Basic Research, Sunnyvale, CA, USA
[c] University of Hawai'i, John A. Burns School of Medicine, Honolulu, HI, USA
[d] German Center for Neurodegenerative Diseases, Bonn, Germany

ABSTRACT

Loss of DJ-1 function contributes to pathogenesis in Parkinson's disease. Here, we investigate the impact of aging and DJ-1 deficiency in transgenic mice. Ventral midbrain from young DJ-1-deficient mice revealed no change in 4-hydroxy-2-nonenal (4-HNE), but HSP60, HSP40 and striatal dopamine turnover were significantly elevated compared to wildtype. In aged mice, the chaperone response observed in wildtype animals was absent from DJ-1-deficient transgenics, and nigral 4-HNE immunoreactivity was enhanced. These changes were concomitant with increased striatal dopamine levels and uptake. Thus, increased oxidants and diminished protein quality control may contribute to nigral oxidative damage with aging in the model.

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1. Introduction

The link between DJ-1 and Parkinson's disease (PD) was first established with the discovery of two homozygous mutations in the DJ-1 gene in kindreds with autosomal recessive early-onset parkinsonism [1]. Studies of the cellular role of DJ-1 have shown pleomorphic function, which is oxidation dependent [2–4]. The protein is involved in transcriptional and translational regulation [3,5–8] as well as apoptosis and autophagy [9–15] and associates with synaptic vesicles, mitochondria and nuclei [16–21]. DJ-1 has also been implicated as a protease [22–26], although not all studies support the idea of DJ-1-induced proteolysis [27,28]. An interesting regulatory feature of DJ-1 involves its oxidative modification. DJ-1, in an oxidized form, exhibits chaperone activity and is able to prevent the fibrillation of the protein alpha-synuclein *in vitro*, an effect not observed with reduced forms [2,4]. While these represent possible mechanisms by which DJ-1 may contribute to neurodegener-ation, most studies have focused on the sensitivity of DJ-1 to oxidative challenge.

Models of DJ-1 deficiency have demonstrated increased suscep-tibility to oxidative injury, providing evidence that DJ-1 is key in the cellular response to stress [29–33]. After oxidative challenge, DJ-1 undergoes an acidic pI shift which is: (i) transient, (ii) present in human PD brain and (iii) due in part to the oxidation of a cys-teine residue at position 106 (C106) [34,35]. The formation of sul-finic acid at C106 via side chain oxidation correlates with the elimination of hydrogen peroxide in vitro, indicating an anti-oxi-dant function of the protein, and a redistribution of the protein to the mitochondrial inner membrane [34,36–38]. Cell culture and animal models of DJ-1-deficiency also demonstrate abnormal mitochondrial membrane potential and morphology [11,17,39–45].

Previous studies have utilized naïve aged (i.e. non-toxicant ex-posed) DJ-1 knockout mice to evaluate the relationship between DJ-1 and aging, with inconsistent outcomes. While striatal altera-tions in dopamine content and protein carbonylation have been re-ported with aging in null DJ-1 line [46], no evidence of oxidative injury was observed in a distinct knockout mouse [47]. Conversely, overexpression of DJ-1 has been shown to protect against toxicant-induced injury within the nigrostriatal pathway and in cultured

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* Corresponding author. Address: SRI International, Center for Health Sciences, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA.
 E-mail address: amy.manningbog@sri.com (A.B. Manning-Boğ).
 [1] These authors contributed equally to this work.

dopaminergic neurons [30,37,48–52]. Here we evaluate the impact of reduced DJ-1 expression on striatal dopaminergic tone and markers of nigrostriatal oxidative injury as a consequence of aging, using DJ-1-deficient mice [53].

2. Materials and methods

2.1. Animals

Mice were maintained on a 12-h light–dark cycle and given free access to food and drinking water as well as standard environmental enrichment. All animal procedures and animal care methods were approved by the Institutional Animal Care and Usage Committees for SRI International and The Parkinson's Institute. Experiments in this study were conducted and reported in accordance with EU Directive 2010/63/EU for experimental animal use.

No protein was detected in brain from genotypic null mice as previously described [31]. Male and female 3-4 month old littermate mice from DJ-1-deficient ($n = 9$–14) and DJ-1+/+ control ($n = 9$–12) cohorts, along with DJ-1-deficient and wildtype littermate mice aged 15–18 months ($n = 6$–9 per genotype), were euthanized by cervical dislocation. Brain regions were dissected on ice and utilized for biochemical and histological analyses. Experiments were performed by investigators blinded to group.

2.2. Neurochemistry

Striatal samples were dissected at the level of the anterior commissure. Samples were placed in 1 ml ice-cold 0.4 M perchloric acid, sonicated and centrifuged [53]. The supernatant was collected for measurement of dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by reverse phase HPLC with electrochemical detection (Coulochem II detector; ESA, Chelmsford, MA) [54]. Total protein was determined using the Lowry method.

2.3. ^{3}H-dopamine uptake

Striatal synaptosomes were prepared from fresh tissue homogenized in 0.3 M sucrose and incubated in assay buffer with [^{3}H]-DA (20 nM; PerkinElmer) as described [53]. Synaptosomes were incubated for 10 min at 37 °C, and then uptake was terminated by the addition of ice-cold buffer and rapid vacuum filtration. Filters were washed, air-dried and placed in Econoscint (Fisher Scientific, Pittsburgh, PA) for scintillation counting. Uptake rate was calculated as total–non-specific uptake, with non-specific uptake determined with 10 μM nomifensine. Protein concentration was measured by the Lowry method.

2.4. Immunohistochemistry

The mid- and hind-brain were post-fixed in 4% paraformaldehyde in PBS. Brain blocks were subsequently cryoprotected in 10% and 30% sucrose for at least 4 h or until sunk and subsequently frozen and maintained at −80 °C. Coronal sections (40 μm) were cut and collected serially into tubes containing cryoprotectant solution and stored at −20 °C until further use. Immunolabeling with primary antibodies against rabbit or sheep tyrosine hydroxylase ((TH);1:600, Pel Freez Biologicals) and/or rabbit 4-HNE (1:2000, Calbiochem) was performed overnight at 4 °C. For profile counting, sections were incubated successively in rabbit anti-4-HNE, biotinylated anti-rabbit secondary antibody, and HRP-linked avidin:biotin complex. Reactivity was visualized with DAB (Vector Labs), and sections were then lightly counterstained with 0.5% Cresyl violet. Sections containing third nerve were used for analyses,

as well as the sections 240 μm rostral and caudal to that section. Counting was performed by two investigators blinded to age and genotype. The substantia nigra was delineated at low magnification and sampled at high magnification using the optical fractionator technique [55]. For confocal microscopy, sections were incubated with sheep anti-TH and rabbit anti-4-HNE primary antibodies followed by FITC-conjugated goat anti-rabbit and Cy3-conjugated goat anti-sheep secondary antibodies, mounted in Vectashield and coverslipped.

2.5. Stereological analysis

Serial coronal sections of mouse midbrain containing the entire extent of the substantia nigra were cut on a cryostat at 40 μm, collected in cryopreservative and stored. For stereological cell counts, every 6th tissue section, encompassing the entire substantia nigra, was probed for TH immunoreactivity and visualized with DAB as described above. After sections were mounted to slides and air-dried overnight, tissues were cleared, counterstained in 0.5% Cresyl violet for 5 min, dehydrated and coverslipped in DePeX.

Stereological analysis was performed using an Olympus BH2 microscope with a motorized X-Y stage linked to a computer-assisted stereological system (Castgrid; Olympus, Albertslund, Denmark). The substantia nigra pars compacta was delineated at low magnification; the pars compacta subregion was distinguished from pars reticulata based on cell morphology, cytoarchitecture and orientation according to previous studies [53,56]. Sections were systematically sampled at 100× magnification. They were examined in the z direction through the optical plane with the dissector at 8 μm and a guard range of 2 μm. The total number of dopaminergic neurons was estimated using the optical fractionator technique for an unbiased estimate of total number independent of size, shape and shrinkage.

2.6. Immunoblotting

Following sonication of striatum in Tris–EDTA buffer with protease and phosphatase inhibitors (Sigma), samples were centrifuged, and the supernatant aspirated. Protein concentration was measured by BCA assay (Pierce), and supernatant proteins separated by SDS–PAGE (12% Tris–glycine, Invitrogen/Life Technologies, Carlsbad, CA) then transferred to nitrocellulose. Blots were blocked in 5% milk and incubated overnight with chicken anti-glyceraldehyde 3-phosphate dehydrogenase (1:2000, GAPDH; Chemicon/Millipore, Billerica, MA), mouse anti-HSP40 (1:1000, Assay Designs/Stressgen, Enzo Life Sciences, Farmingdale, NY), mouse anti-HSP60 (1:1000, Assay Designs/Stressgen), and mouse anti-HSP70 (1:800, Assay Designs/Stressgen). Next, appropriate peroxidase-conjugated secondary antibodies were applied, and signal visualized following incubation with chemiluminescent substrate (Thermo Fisher Scientific, Rockford, IL). After exposure to CL-XPosure Film (Thermo Scientific), immunoreactivity was quantified by Image J software.

2.7. Statistical analyses

Differences among means were analyzed using two-way analysis of variance (ANOVA) when comparing differences between genotype and age. Bonferroni's post hoc analysis was employed when differences were observed in ANOVA testing ($P \leqslant 0.05$).

3. Results

We evaluated whether changes in nigrostriatal dopaminergic tone and oxidative stress occur as a consequence of aging in a

mouse model of DJ-1 deficiency. Cohorts were defined as young and old based on age (i.e. 3–4 or 15–18 months, respectively) and by genotype (i.e. DJ-1-deficient or wildtype littermate).

Our laboratory and others have demonstrated functional alterations in dopaminergic transmission in DJ-1-deficient mice that correlate with deficits in motor behavior, and these are exacerbated with aging [53,57]. One mechanism thought to contribute to these changes specifically involves an increase in DA transporter reuptake activity; no significant alterations in total DA or the metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels have been reported in striatal homogenates from young genotypic null mice and wildtype littermates [30,31,33,46,58]. In the present study, we confirmed no differences due to genotype in DA, DOPAC and HVA levels in young mice (Fig. 1A), however, further analyses revealed a modest, but significant, increase in DA turnover (21.8%) was noted in the 3–4 month-old DJ-1-deficient vs. wildtype littermates (Fig. 1C). Animals aged to 15–18 months demonstrated DA levels significantly higher (47.6%) in the aged DJ-1-deficient group (Fig. 1B), but DOPAC and HVA were not altered as a consequence of genotype (Fig. 1B). Consequently, DA turnover was significantly decreased in aged animals lacking DJ-1 (Fig. 1C), along with increased DA uptake in striatal synaptosomes vs. wildtype mice (Fig. 1D).

Since enhanced activity of the DA transporter and increases in DA turnover can lead to increased intracellular oxidative stress and mitochondrial dysfunction, we evaluated markers of oxidative injury in the substantia nigra. A reactive aldehyde by-product of lipid peroxidation, 4-HNE, was evaluated using histological assessment as previously described [59]. In young mice, little 4-HNE immunoreactivity was apparent within nigral neurons regardless of genotype (Fig. 2A). To assess a mitochondrial response indicative of oxidative stress, levels of the mitochondrial chaperone HSP60 and HSP40 were evaluated; both proteins were significantly augmented, 49.3% and 39.8%, respectively, in ventral mesencephalon homogenates from DJ-1-deficient transgenics over wildtype littermates (Fig. 2B, C). No difference due to genotype was noted in the cytoplasmic chaperone HSP70 in young mice, however (Fig. 2G). In the aged cohort, a robust increase in 4-HNE immunoreactivity was apparent in substantia nigra of DJ-1-deficient vs. wildtype mice (Fig. 2D). While no difference between genotypes was observed in HSP60 and HSP40 levels in ventral mesencephalon (Fig. 2E, F), a marked depletion in HSP70 was noted in the aged animals lacking DJ-1 (Fig. 2H).

To gain insight into whether the lack of difference in chaperone expression in aged ventral midbrain was due to a significant change in the wildtype littermates or a decline in expression with DJ-1 deficiency, we compared chaperone expression in young vs.
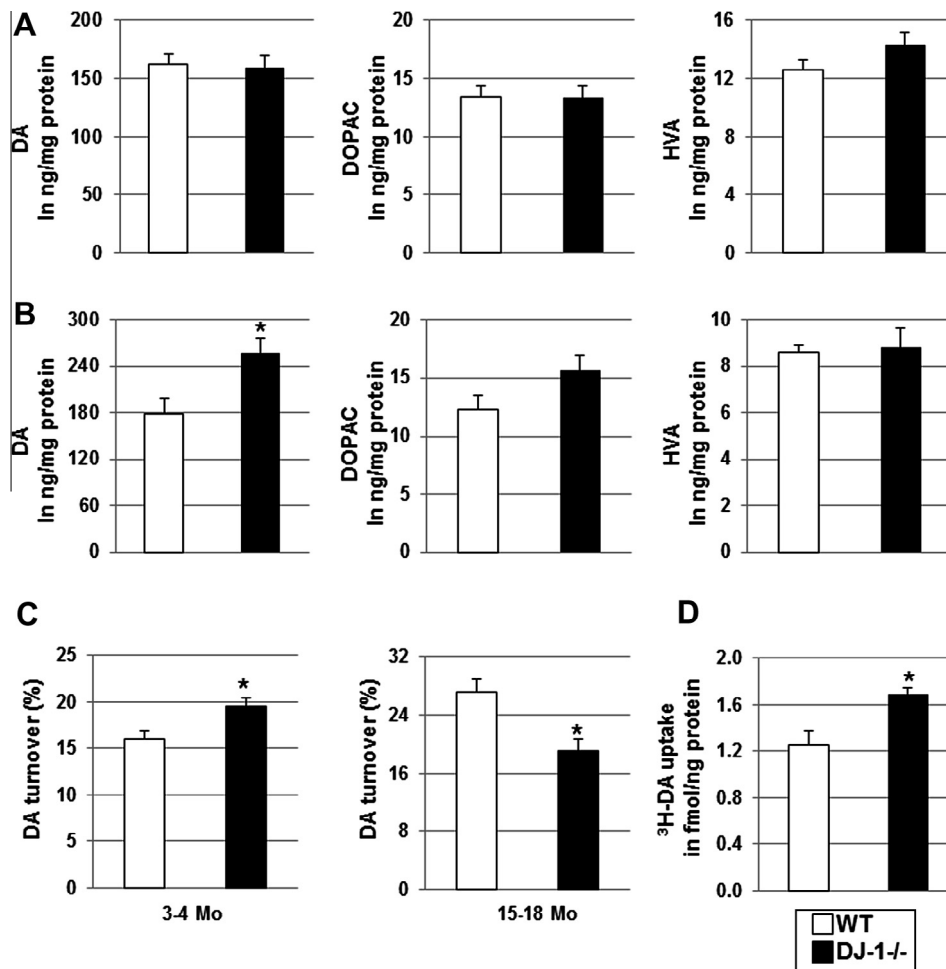

Fig. 1. Neurochemical analyses of striatal DA and metabolites by HPLC in young and aged DJ-1-deficient vs. WT mice. (A) Striatal supernatant from young WT (open bars) and DJ-1-deficient (black bars) was used to measure DA, DOPAC and HVA by HPLC. (B) DA and metabolites measurements were performed in striatum from aged mice (*; $P < 0.05$). (C) DA turnover was calculated as a percentage of total metabolites (HVA + DOPAC) over total DA measured and compared in young (left panel) and aged (right panel) DJ-1-deficient and WT mice (*; $P < 0.01$). (D) ^{3}H-DA uptake was measured in synaptosomes from aged WT (open bar) and DJ-1-deficient (black bar) mice; (*;$P < 0.05$). Data are shown as mean ± S.E.M.
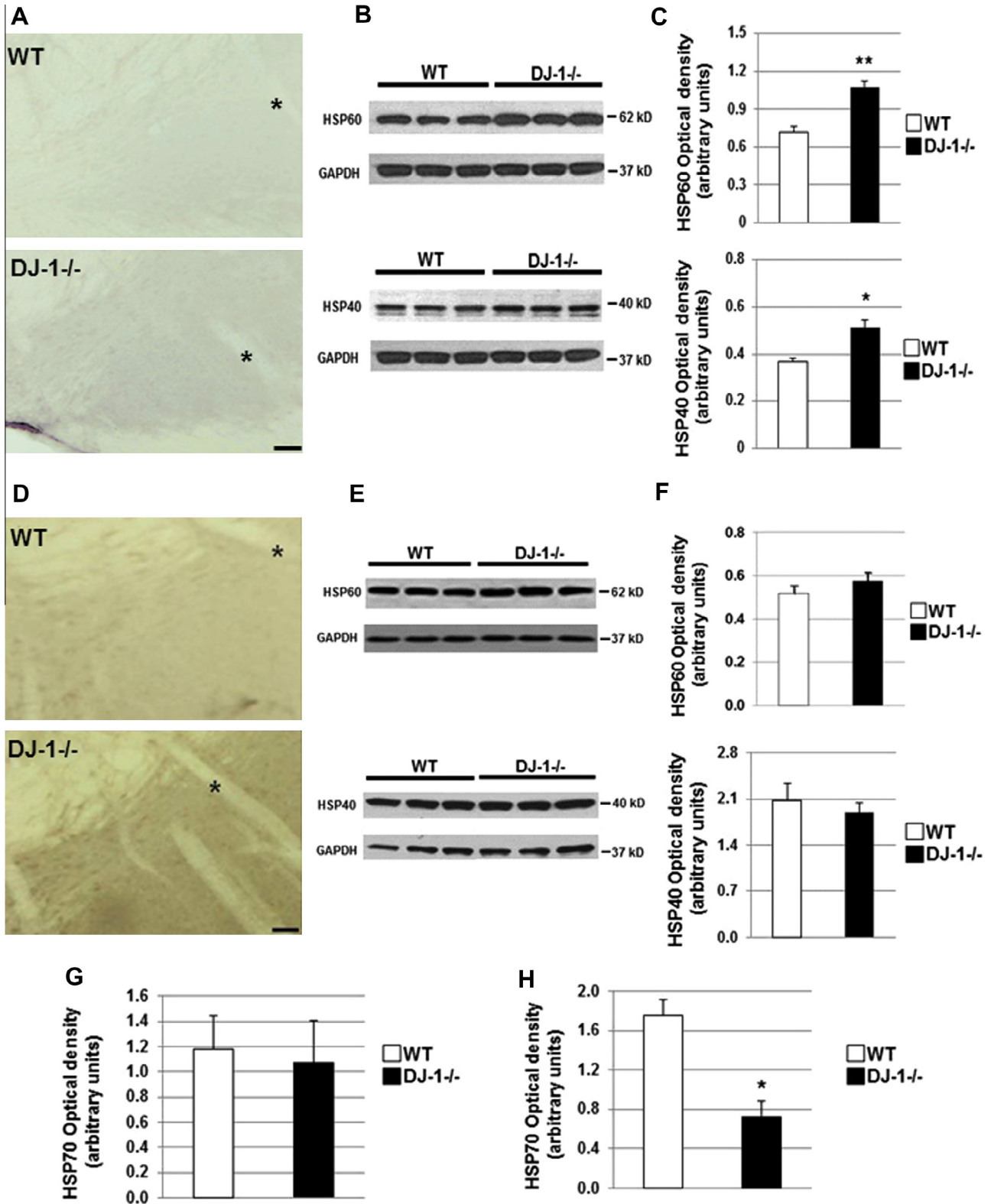
Fig. 2. Effect of genotype on lipid peroxidation and chaperone levels in young and aged DJ-1-deficient vs. WT mice. (A) Coronal midbrain sections containing substantia nigra were immunostained for the lipid peroxidation product 4-HNE in young WT and DJ-1-deficient mice. * denotes third nerve. (B) Western blot analyses were used to assess HSP60 and HSP40 in ventral mesencephalon from young mice. (C) Optical density of immunoreactivity was measured in Western blots from young WT (open bars) vs. DJ-1-deficient (black bars) mice (*, $P < 0.05$; **, $P < 0.01$). (D) Coronal midbrain sections containing substantia nigra were immunostained for 4-HNE in aged WT and DJ-1-deficient mice. * denotes third nerve. (E) Western blot analyses were used to assess HSP60 and HSP40 in ventral mesencephalon from aged mice. (F) Optical density of immunoreactivity was measured in Western blots from aged WT (open bars) vs. DJ-1-deficient (black bars) mice. (G) Optical density of HSP70 immunoreactivity was measured in Western blots from ventral mesencephalon of young WT (open bar) vs. DJ-1-deficient (black bar) mice. (H) Optical density of HSP70 immunoreactivity was measured in Western blots from ventral mesencephalon of aged WT (open bar) vs. DJ-1-deficient (black bar) mice. (*, $P < 0.05$). Bars = 40 μn. Data are shown as mean ± S.E.M.

aged mice of the same genotype. Interestingly, HSP60 and HSP70 expression were markedly increased with age in wildtype ventral mesencephalon whereas this effect was absent in the aged DJ-1-deficient mice (Fig. 3A, C). Conversely, HSP40 expression was unchanged in the samples from wildtype mice with aging, but was significantly decreased in the aged DJ-1-deficient tissue (Fig. 3B).

To verify that the oxidative damage was specific for the dopaminergic cell population, we performed dual label immunohistochemistry using anti-TH and anti-4-HNE. Examination using confocal microscopy in midbrain sections from naïve DJ-1-deficient and wildtype littermate mice revealed that immunoreactivity for 4-HNE co-localized with the TH-positive neuronal population (Fig. 4A), providing evidence for lipid peroxidation within nigral dopaminergic cells. In order to quantitatively assess any differences, we performed profile counting of 4-HNE-positive cells in the substantia nigra pars compacta following previously reported

criteria [60]. In line with qualitative analyses (Fig. 2A), relatively few 4-HNE-positive profiles per section were detected in young mice with no difference between cohorts separated for genotype (Fig. 4B). An increase in 4-HNE-positive neuronal profiles per section was observed in aged vs. young wildtype substantia nigra (Fig. 4B). Interestingly, this effect was exacerbated in nigral neurons from aged DJ-1-deficient mice. Significantly higher numbers of 4-HNE-immunoreactive profiles were detected in this cohort compared to aged wildtype littermates (21.8 ± 3.1 vs. 9.7 ± 3.7), revealing a 2.2-fold increase in this marker of oxidative injury. Because protein carbonylation has been reported in extra nigral regions in PD brain [61], we evaluated if changes in 4-HNE were apparent in the ventral tegmental area. No increase in 4-HNE reactivity due to genotype was noted in this region from aged animals (Fig. 4C). Lastly, we assessed whether increased nigral lipid peroxidation in aged DJ-1-deficient mice was associated with frank cell death using unbiased stereological cell counting. No significant
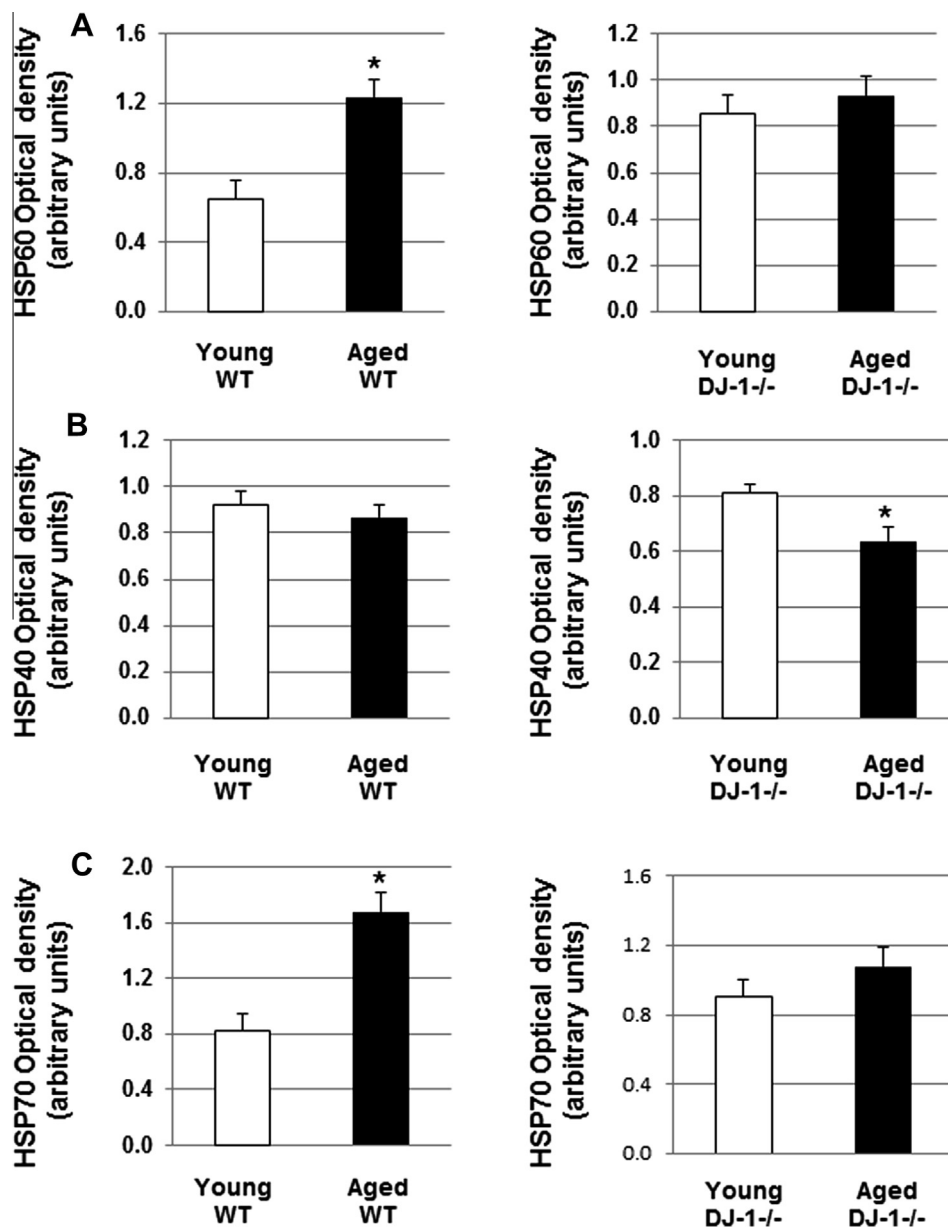

Fig. 3. Effect of aging on the mitochondrial and cytosolic chaperones in ventral mesencephalon from DJ-1-deficient and WT mice. Western blot analyses were used to assess the effects of aging on HSP60 (A), HSP40 (B) and HSP70 (C) levels in ventral mesencephalon from in young (open bars) vs. aged (black bars) mice from both WT (left panel) and DJ-1-deficient (right panel) genotypes. (*, P < 0.05). Data are shown as mean ± S.E.M.
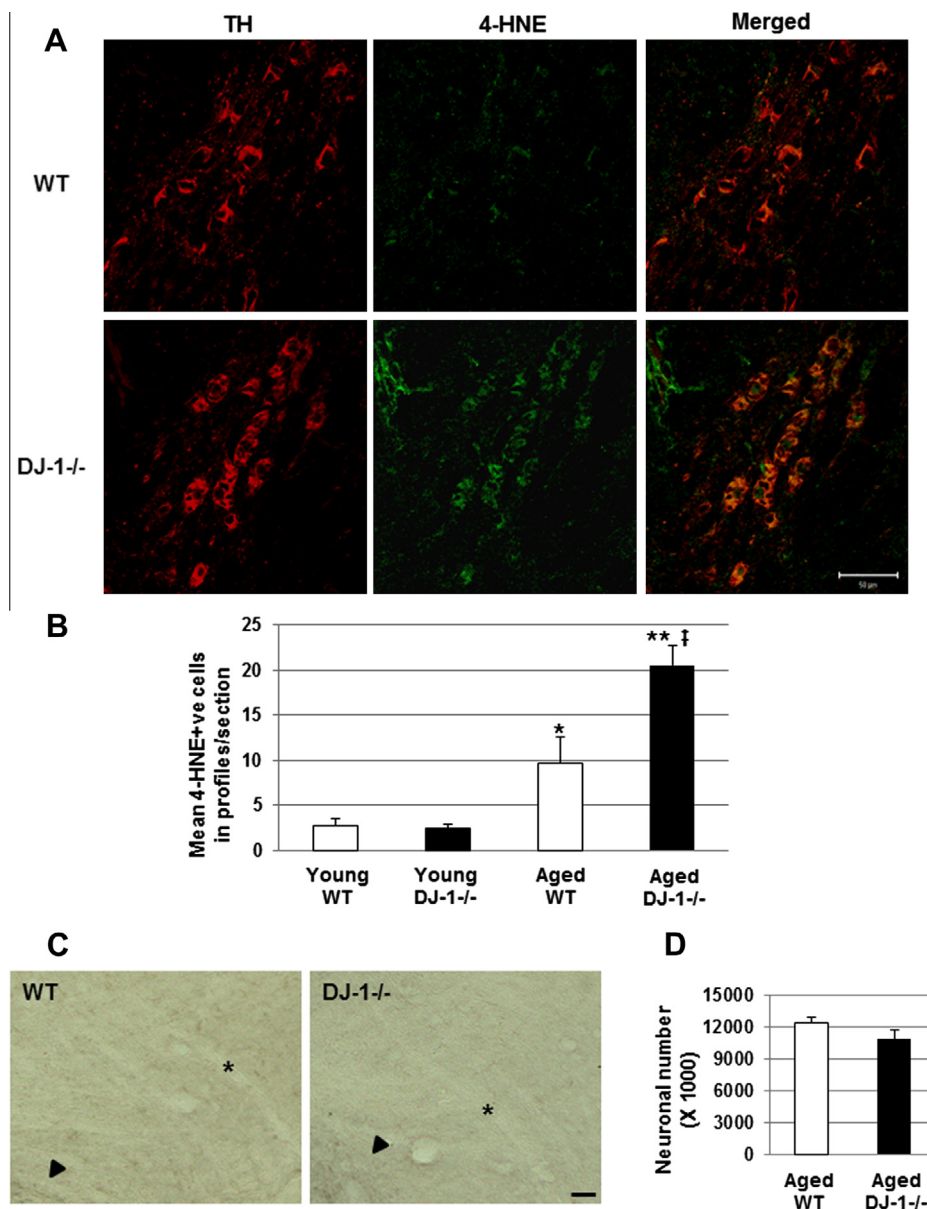
Fig. 4. Lipid peroxidation in ventral midbrain and unbiased stereological cell counting of nigral dopaminergic neurons in DJ-1-deficient vs. WT mice. (A) Dual-label immunohistochemistry for 4-HNE (FITC) and TH (Cy3) was performed in sections in the substantia nigra from aged DJ-1-deficient vs. WT mice. Merged panel (right) demonstrates colocalization of immunoreactivities. (B) Quantitative assessment of lipid peroxidation by 4-HNE-positive neuronal profiles in substantia nigra from young and aged DJ-1-deficient and WT mice was performed. (* aged vs. young WT, $P < 0.05$;** aged DJ-1 vs. young DJ-1, $P < 0.01$; ‡ aged DJ-1 vs. aged WT; $P < 0.05$). (C) Coronal midbrain sections containing the ventral tegmental area were immunostained for the lipid peroxidation product 4-HNE in aged WT and DJ-1-deficient mice. * denotes third nerve. Arrowhead denotes interpeduncular nucleus. (D) Unbiased stereological cell counting of Nissl-positive neurons in the substantia nigra was performed in aged DJ-1-deficient (black bar) vs. WT (open bar) mice. Bars = 50 μm. Data are shown as mean ± S.E.M.

difference in TH-positive (data not shown) or Nissl-positive neurons in the substantia nigra was observed in aged animals (Fig. 4D).

4. Discussion

We evaluated young and aged cohorts from a line of DJ-1-deficient vs. wildtype littermates to gain insight into DJ-1-related mechanisms that contribute to nigrostriatal vulnerability [31]. As anticipated, striatal DA and its metabolites did not change significantly in young DJ-1-deficient mice (Fig. 1A), with very few 4-HNE-immunoreactive profiles observed in nigral neurons (Figs. 2A and 4B). However, a significant increase in striatal DA turnover was noted in young mice lacking DJ-1 vs. wildtype controls (Fig. 1C) and was concomitant with marked elevations of the chaperones

HSP60 and HSP40, but not HSP70, in the ventral mesencephalon (Fig. 2B). These findings demonstrate that, in the absence of sufficient DJ-1 in young mice, alterations in striatal DA turnover occur, and these changes are accompanied by evidence for mitochondrial stress, but not overt injury such as lipid peroxidation.

In aged DJ-1-deficient vs. control mice, a distinct picture emerges implying aberrant age-related responses in the nigrostriatal pathway of animals lacking DJ-1. Enhanced striatal DA but unchanged DOPAC and HVA values were detected (Fig. 1B) resulting in reduced DA turnover in the transgenics (Fig. 1C). Furthermore, DA uptake was significantly elevated in the DJ-1-deficient mice (Fig. 1D). Together, these findings suggest that with aging: (i) abnormalities in striatal dopaminergic neurotransmission due to insufficient DJ-1 persist, and (ii) DJ-1 contributes to presynaptic

DA tone not only via modulating DA reuptake but through modulation of striatal DA levels.

The findings reported here provide insight into DJ-1-related processes that may contribute to nigrostriatal injury with aging (i.e. enhanced levels of reactive DA metabolites and toxic by-products). Although our laboratory and others found no difference in total striatal DA levels due to genotype in young DJ-1-deficient mice (Fig. 1A) [53,57,58,62,63], the significant increase in DA turnover shown here (Fig. 1C) suggests a condition that favors increased generation of reactive oxygen species (ROS) in young animals [64,65]. The precise pathway(s) underlying altered turnover due to DJ-1 insufficiency is currently not known. Previous studies have shown that DJ-1 impacts TH transcription [66–68], and, in two knockout lines on the highly oxidative stress-sensitive C57BL/6 background, lack of DJ-1 leads to a reduced number of nigral neurons with TH phenotype [63,69]. However, these reports did not extend to enzymes involved in DA catabolism. Others have reported that parkin diminishes monoamine oxidase (MAO) transcript levels and thus can limit oxidative stress through control over MAO-mediated ROS production [70,71]. Given DJ-1-mediated effects on transcription and translation [72], and reports of functional interactions between DJ-1 and parkin [15,73], the possibility of DJ-1-related modulation of DA catabolic enzymes exists, and this warrants investigation in future studies.

Given that our lab and others have previously shown enhanced DA transporter activity in DJ-1-deficient young mice [53,58], increased DA turnover is not entirely surprising. Though the elevation in DA turnover in young DJ-1-deficient animals was modest (21.8%), a substantial increase in two molecular chaperones, HSP60 and HSP40, was concomitantly detected in the ventral midbrain from transgenics (Fig. 2B). HSP60 is a mitochondrial matrix protein that serves as a chaperone to mitigate aberrant folding [74]. Its expression has been shown to increase under conditions of oxidative challenge [75,76], including those that enhance DA catabolism [76], but can diminish in pathological states [77–79]. Similarly, HSP40 is also involved in the response to stress-induced protein misfolding [80–82], acts as a co-chaperone with HSP70 [83], and is up-regulated following exposure to oxidative toxicants that increase DA turnover [82] and consequently intracellular hydrogen peroxide [64,84]. Therefore, it is possible that the observed increases in nigral HSP expression in young mice are, at least in part, in response to elevated turnover of DA in the striatum. Importantly, these chaperones may serve as early indicators of mitochondrial dysfunction that leads to nigral injury.

Interestingly, HSP70, a cytoplasmic chaperone, were not altered due to genotype in ventral mesencephalon from young animals (Fig. 2G). One possibility is that elevated levels of the co-chaperone HSP40 are sufficient to increase HSP70 ATPase activity and chaperone function [83]. Furthermore, although oxidative challenge from increased DA turnover was present in young transgenics, there was a lack of detectable 4-HNE (Fig. 2A). This lipid peroxidation product can promote induction of HSP70 expression by a transcription factor heat shock factor 1 (HSF 1)-dependent mechanism. Under normal conditions, HSF 1 binds with HSP70, an interaction which maintains HSF 1 in the cytoplasm. However, with oxidative injury, 4-HNE disrupts this protein-protein interaction allowing HSF 1 to translocate into the nucleus, promoting transcription of the gene encoding for HSP70 [85]. It may be that in the young transgenics, as in other models [86], mitochondrial dysfunction is present and occurs prior to cytoplasmic changes that elicit an HSP70 response such as cytoplasmic protein misfolding.

Increased striatal DA values in aged DJ-1-deficient mice are consistent with findings of Chen et al. (2005); this, in combination with an age-related decline in HVA (Fig. 1B), caused a significant reduction in DA turnover in DJ-1-deficient striatum (Fig. 1C). Given that DJ-1 acts as a transcriptional activator for TH [67,68], the find-

ing of increased striatal DA with aging seems somewhat counterintuitive. It is likely that the enhanced DA transporter activity observed in older DJ-1-deficient mice contributes to this effect (Fig. 1D), as increased reuptake of DA would be anticipated to increase neurotransmitter levels within the tissue [57]. By example, in DA transporter-knockout mice, reuptake is attenuated, and total tissue levels of DA are reduced in the striatum [87]. Another plausible explanation is that the activities of DA metabolic enzymes, including TH, aromatic amino acid decarboxylase [88,89] and catechol-O-methyltransferase [90] may be affected by oxidative challenge. In response to the herbicide paraquat or the dopaminergic toxin 6-hydroxydopamine, for example, striatal TH activity in rodents is upregulated [91–93]. Further, recent studies have shown that the lack of DJ-1enhances the activity of extracellular signal-related kinase (ERK) [94], which can phosphorylate TH; it is possible that DJ-1-related elevation in ERK activity may promote increased TH modification and consequently activity in DJ-1-deficient striatum.

It is important to point out that the enhanced striatal DA uptake in the aged DJ-1-deficient mice (Fig. 1D), in combination with increased DA levels, may promote more robust 4-HNE staining and profile counts observed in nigral neurons from the transgenics vs. wildtype mice (Fig. 4B). Recent work by Lev and colleagues revealed that DJ-1 regulates expression of the vesicular monoamine transporter-2 (VMAT2) which removes reactive DA from the cytoplasm and sequesters the neurotransmitter in synaptic vesicles [95]. Thus, it is possible that VMAT2 deficiency in the absence of DJ-1 may also contribute to lipid peroxidation through increased levels of cytoplasmic DA. Our detection of nigral dopaminergic 4-HNE deposition is divergent from an earlier study which did not detect frank oxidative damage with aging in a distinct DJ-1 knockout line [96]. Subsequent work from this group, however, confirmed elevated ROS production with DJ-1 deficiency [42]. These findings, along with the results presented here, are consistent with the idea that insufficient DJ-1 leads to impairments in mitochondrial uncoupling proteins that respond to oxidative challenge [42,97]. Although cells were damaged, there was no significant nigral dopaminergic cell death (Fig. 4D). Thus, these neurons may have a 'silent lesion' and are likely more vulnerable to subsequent insult (i.e. risk factors for sporadic PD).

Age-related increases in the chaperones HSP60 and HSP70 observed in wildtype mice were absent from the DJ-deficient mice (Fig. 3A, C). Enhanced HSP60 and HSP70 in mammalian brain have been reported with senescence [98]; however, the diminished HSP60 levels in the aged transgenics mimics an effect noted previously in disease states and models involving mitochondrial dysfunction (Fig. 3A) [77–79]. Our data further implicate a modulatory role for DJ-1 in HSP-mediated protein quality control. Reduced HSP levels may be due to chronic inhibition of the Nrf2 transcription factor response caused by DJ-1 deficiency. Normally, DJ-1 binds to Nrf2 and prevents its interaction with Keap1. Keap1–Nrf2 binding signals ubiquitination of Nrf2 resulting in its degradation through the proteasome and reducing expression of anti-oxidant molecules and HSPs including HSP40 and HSP70 [52]. Indeed, the lack of an Nrf2-mediated response has been observed in another model of DJ-1 insufficiency and contributes to deficits in protein quality control [99].

While decreases in proteasomal subunits have been reported in another DJ-1 knockout mouse [100], this is the first study to demonstrate that the lack of DJ-1 attenuates age-induced HSP expression. Mitochondrial and cytoplasmic proteins in nigral neurons from aged DJ-1-deficient mice, thus, are likely more susceptible to lipid peroxidation and aberrant protein folding. Taken together, the findings support the idea that changes in DJ-1 levels and function(s), due to either genetic variation in familial parkinsonism or irreversible oxidation of DJ-1 observed in the sporadic disease

[101], render neurons more vulnerable to protein handling abnormalities, oxidative damage, and ultimately nigrostriatal lesioning.

Acknowledgments

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