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DJ-1 mRNA anatomical localization and cell type identification in the mouse brain

Zhiguo Xie[1,2], **Xiaoxi Zhuang**[2], and **Linan Chen**[2,*]

[1] State Key Laboratory of Medical Genetics of China, Xiangya Hospital, Central South University, Changsha, Hunan, China

[2] Department of Neurobiology, The University of Chicago, Chicago, Illinois, USA

Abstract

Mutations in DJ-1 cause familial Parkinson's disease (PD). The expression pattern of DJ-1 in the brain remains controversial. In the present study, we used DJ-1 deficient mice as negative controls and examined DJ-1 mRNA expression in mouse brains. By sequential double labeling on the same sections, *in situ* hybridization of DJ-1 mRNA was followed by immunofluorescence detection for cell type markers. We found that DJ-1 mRNA was expressed in the majority of neurons in all brain areas examined. In particular, all dopamine neurons in the ventral midbrain expressed DJ-1 mRNA. Interestingly, the choroid plexus and ependymal cells lining the ventricles were the only non-neuronal regions strongly expressing DJ-1 mRNA. However, DJ-1 mRNA was not detected in astrocytes. DJ-1 mRNA expression in all nigra dopamine neurons but not in astrocytes suggests that its potential neuroprotective role could be cell-autonomous. The fact that DJ-1 expression is not restricted to substantia nigra dopamine neurons suggests that DJ-1 mutations may collaborate with other predisposing factors to cause the relatively selective dopamine neuron degeneration in Parkinson's disease.

Introduction

Several missense and deletion mutations of DJ-1 cause early onset familial Parkinson's disease. Several studies suggested DJ-1 may be involved in oxidative sensoring [13], ubiquitin proteasome system [20] and post-transcriptional regulation [9].

The expression pattern of DJ-1 in the brain remains controversial. It is unclear whether DJ-1 is expressed in astrocytes or neurons and whether DJ-1 is expressed in dopamine neurons. Three published studies on human brains found DJ-1 protein was localized in astrocytes but not in neurons using several antibodies [2,3,14]. However, two other groups found DJ-1 protein was present mostly in neurons and less in astrocytes in human brains [4,17]. In the mouse brains, whether DJ-1 protein is present in astrocytes has also raised controversies [1,3,15,18, 19]. *In situ* hybridization studies suggested DJ-1 mRNA expression in neuron-like but not astrocyte-like cells judged solely by the morphologies of DJ-1 positive cells [1,5,18]. Two study even found that DJ-1 protein was not present in dopamine neurons in rats [10,19].

*Corresponding author: Linan Chen, M.D. Ph.D., 924 E. 57th St. R222, Knapp Center, Department of Neurobiology, The University of Chicago, Chicago, Illinois, USA 60637, lichen@bsd.uchicago.edu., Tel.: 1-773-834-9062, Fax: 1-773-834-3808.

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In the present study, our goal was to characterize DJ-1 mRNA expression pattern in the mouse brain and determine whether astrocytes and neurons, especially dopamine neurons, express DJ-1 mRNA. We used DJ-1 deficient mice as negative controls to ensure the specificity of *in situ* hybridization. Moreover, we took advantage of superior cellular resolution of non-radioactive *in situ* hybridization technique in combination with immunofluorescence staining for cell type specific markers.

Materials and methods

All our data were obtained with the inclusion of DJ-1 deficient brain sections to control for staining specificity. The lack of DJ-1 mRNA and protein in our DJ-1 deficient mice brains were confirmed by RT-PCR (Fig. 1A) and western blot. Signet rabbit anti-DJ-1 antibody (Covance, Emeryville) and Santa Cruz rabbit anti-DJ-1 antibody (C-16, Santa Cruz Biotechnology, Santa Cruz), recognized one major 20 kD mouse DJ-1 band in wild type mice, which was absent in DJ-1 deficient mice (Fig. 1B).

Two to three-month old C57BL/6J mice and DJ-1 deficient mice [5] were used for the present study. The animals were housed in a 12:12 h light/dark cycle with *ad libitum* food and water. All animal procedures were approved by the Institutional Animal Care and Usage Committee of The University of Chicago. Mice were transcardially perfused with 4% paraformaldehyde. A full-length DJ-1 cDNA (NM_020569) was labeled with nonradioactive DIG RNA labeling kit (Roche, Indianapolis). For nonradioactive *in situ* hybridization, sections (20μm) were treated with 25μg/ml Proteinase K (Roche, Indianapolis) for 30 minutes, then incubated with labeled probe for 16 hours at 63°C followed by RNase A digestion (50μg/ml, Ambion, Austin) for 30 minutes at 37 °C. DJ-1 mRNA signal was detected with Anti-Digoxigenin-AP (Roche, 1:5000) and visualized by NBT/BCIP. All images were captured under Zeiss Stemi SV6 stereo microscope at constant exposure and magnification and analyzed with NIH ImageJ software to assess relative DJ-1 mRNA expression level. Three thresholds were set and labeling intensities were rated (Table 1).

To identify cellular types expressing DJ-1 mRNA, DJ-1 *in situ* hybridization was followed by immunofluorescence staining on the same sections. Tyramide signal amplification (TSA) technique was used to amplify the immunofluorescence signal. Briefly, after *in situ* hybridization, sections were blocked and detected with following antibodies: mouse anti-NeuN (1:500, Millipore, Bedford), mouse anti-TH (1:500, BD Transduction, Lexington) and rabbit anti-GFAP (1:500, DAKO, Carpinteria). Sections were then incubated with a biotinylated secondary antibody followed by peroxidase conjugated avidin-biotin complex (Vector Laboratories, Burlingame). TSA reagent (PerkinElmer, Boston) was used for visualization under Zeiss Axioplan 2 fluorescence microscope.

To quantify the percentage of NeuN or TH positive cells that express DJ-1 mRNA, pictures were taken under 20× objective lens at three randomly picked locations in each brain regions. All NeuN or TH positive cells in the entire field were counted manually. The percentage of DJ-1 expression in NeuN or TH positive cells was calculated by dividing all $NeuN^+DJ\text{-}1^+$ cells or $TH^+DJ\text{-}1^+$ cells by all $NeuN^+$ or TH^+ cells, respectively.

Results

We found that DJ-1 mRNA was expressed throughout the mouse brain (Fig. 1C) except the white matter. The relative DJ-1 mRNA expression level in brain was summarized in Table 1. As a negative control, DJ-1 mRNA staining was absent in DJ-1 deficient mouse brains (Fig. 1C).

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In olfactory bulb, DJ-1 mRNA was expressed in all cell layers with the highest expression in mitral layer and the lowest in granular layer (Fig. 1D). In the dorsal striatum, DJ-1 mRNA expression was lower than that of most other regions. DJ-1 positive cells showed neuronal morphology. Most DJ-1 positive cells were likely medium spiny neurons which comprise > 90% of the total striatal neurons. There were few densely labeled cells scattering evenly throughout dorsal striatum (Fig. 1E). The number of these large cells (20–35 μm) ranged from 20 to 40 cells per striatal section, which could be certain types of interneurons [16]. DJ-1 mRNA expression in the cerebral cortex was higher than that in the striatum. DJ-1 expression was seen in all layers of the cortex with the highest expression in layer 5, which contained large pyramidal neurons innervating subcortical regions (Fig. 1G). The ventral midbrain had strong DJ-1 mRNA expression (Fig. 1J). DJ-1 mRNA expression level was comparable between substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). The substantia nigra pars reticulata (SNr) had few DJ-1 positive cells.

To examine the cell type identity of DJ-1 positive cells, we performed sequential double labeling on the same sections. There was almost complete overlap of DJ-1 positive cells with NeuN positive cells (Fig. 2A). To quantify the percentage of DJ-1 expression in neurons, we counted cells positive for DJ-1, NeuN or both in several brain areas. In the frontal cortex, 96.3% cells (473 out of 491 NeuN positive cells) expressed DJ-1 mRNA. Conversely, few DJ-1 positive cells were negative for NeuN. Since most of these NeuN negative cells had typical neuronal morphology, we attributed these cells to extreme loss of NeuN protein due to proteinase K digestion for *in situ* hybridization. In addition to the frontal cortex, NeuN and DJ-1 were highly colocalized in the thalamus, dorsal striatum and amygdala, where more than 95% of NeuN positive cells expressed DJ-1 mRNA (Fig. 2 table). DJ-1 *in situ* hybridization signal was absent in brain sections of DJ-1 deficient mice (Fig. 2B).

GFAP signal apparently showed no colocalization with dark blue staining of DJ-1 mRNA in both gray matter and white matter (Fig. 3A, C). Absence of colocalization of DJ-1 mRNA and GFAP staining was most evident in corpus callosum (Fig. 3A). In corpus callosum, DJ-1 mRNA staining was virtually absent while GFAP positive astrocytes were abundant (Fig. 3A, left panel). Examination under 40× objective lens further confirmed the absence of DJ-1 mRNA in astrocytes (Fig. 3A, right panel). However, DJ-1 mRNA was not exclusively expressed in neurons. Strong expression of DJ-1 mRNA was observed in ependymal cells lining the ventricles and the choroid plexus (Fig. 1I).

Lastly, we tried to address if DJ-1 mRNA was expressed in dopamine neuron in SNc and VTA. Published DJ-1 *in situ* hybridization studies [1,5,18] showed DJ-1 mRNA expression in SNc and VTA but didn't specify whether dopamine neurons expressed DJ-1 mRNA. On the other hand, two studies found absence of DJ-1 protein in dopamine neurons in rat brains [10,19]. Therefore, it is important to demonstrate definitively whether DJ-1 is expressed in dopamine neurons. At low magnification, DJ-1 positive cells were abundant throughout VTA and SNc. Similar distribution patterns were observed between DJ-1 positive cells and TH positive cells (Fig. 4A). In VTA and SNc, virtually all TH positive cells (424 TH positive cells were counted) showed colocalization with DJ-1 positive cells (Fig. 4B and table). Some DJ-1 positive cells didn't display TH immunity, indicated by asterisks in Fig. 4B. These cells had the typical neuronal morphology and scattered throughout the dense TH positive cell populations. Non-dopamine neurons in both VTA and SNc may partially account for these cells [7]. Besides VTA and SNc, we also found nearly all TH positive cells expressed DJ-1 mRNA in the periglomerular region of olfactory bulb, retrorubral area (Fig. 4C, D and table) and hypothalamus (Fig. 4 table). In the locus coeruleus, DJ-1 mRNA was also expressed in nearly all TH positive cells which are presumably norepinephrine neurons (Fig. 4E and table). Therefore, our data clearly established that DJ-1 mRNA was strongly expressed in nearly all dopamine neurons.

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Discussion

This is the first DJ-1 *in situ* hybridization study using DJ-1 deficient mice as negative controls and combining immunofluorescence staining with cell type specific markers. With the highly specific labeling controlled by deficient mice sections, we unequivocally established that DJ-1 mRNA was ubiquitously expressed in nearly all neurons. We also firmly established that virtually all dopamine neurons expressed DJ-1 mRNA in the VTA and SNc. No DJ-1 mRNA expression was detected in astrocytes in adult mouse brains. However, it can not be ruled out that DJ-1 mRNA could be expressed by astrocytes in aged animals and certain experimental conditions, such as stroke [14] and neurotoxin treatment [10]. Cells in the choroid plexus and ependymal cells represent the only non-neuronal cells strongly expressing DJ-1 mRNA in our study. It has been reported that DJ-1 protein is detected in human cerebrospinal fluid (CSF). The choroid plexus and ependymal cells might be one of the sources secreting DJ-1 into CSF.

Previous immunohistochemical studies on DJ-1 protein expression with various DJ-1 antibodies generated conflicting results. Two studies from the same group using three different antibodies, including KAM-S100 from Stressgen, consistently found DJ-1 protein was localized in astrocytes but not in neurons [2,3]. Another group found similar results using KAM-S100 in human brains [14]. However, using the same KAM-S100 antibody, two other groups found DJ-1 protein present mostly in neurons and less in astrocytes, which was further confirmed by several antibodies developed in their own labs [4,17]. In another study on monkey brains, DJ-1 protein was found in astrocytes of cortex but in neurons of striatum and nigra [15]. Specificity of antibodies used in all these studies could strongly influence the experimental results. It is well recognized that antibody specificity could not be guaranteed even with preabsorption control experiments. That could result from crossactivity for the same epitope of different proteins [11]. In addition, DJ-1 could be oxidized and dimerized. It is possible that different forms of DJ-1 could be recognized preferentially by different antibodies. A third possible explanation for the conflicting results is the length of fixation. It was found that longer fixation decreased DJ-1 staining in neurons but increased DJ-1 staining in astrocytes [4].

It is possible that DJ-1 protein expression pattern could be very different from that of mRNA expression. There is evidence that DJ-1 can be secreted into cell culture medium, human CSF and serum and secreted DJ-1 may be picked up by cells [8,10,12]. It was reported that DJ-1 protein was absent in the soma of dopamine neurons [10,19] and selectively enriched in striatal processes [15]. This discrepancy is reminiscent of some axonally transported proteins, such as glutamate decarboxylase and dopamine transporter.

Species difference between primates and rodents has been reported. It could result from the different epitope accessibility in astrocytes and neurons between rodents and primates [15]. However, such species difference of DJ-1 expression was not observed by an *in situ* hybridization study [6]. It is possible that DJ-1 expression could change with aging since most human studies were carried out on postmortem specimen from aged individuals. If this were the case, gradual decrease of DJ-1 protein with age could deprive neurons, especially dopamine neurons, of protection from DJ-1.

In summary, the present study for the first time mapped out DJ-1 mRNA expression pattern and cell type in the mouse brain in great detail and with high specificity. DJ-1's neuronal expression pattern, especially its expression in dopamine neurons, suggests that its potential neuroprotective role could be cell-autonomous. The discrepancy between DJ-1 mRNA expression pattern and reported DJ-1 protein expression pattern suggests that the majority of DJ-1 protein might be axonally transported to terminal regions. Future studies on DJ-1 protein

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expression pattern and cell identity in comparison with our mRNA expression data could offer important insights to the normal function of DJ-1 as well as its role in the pathogenesis of PD.

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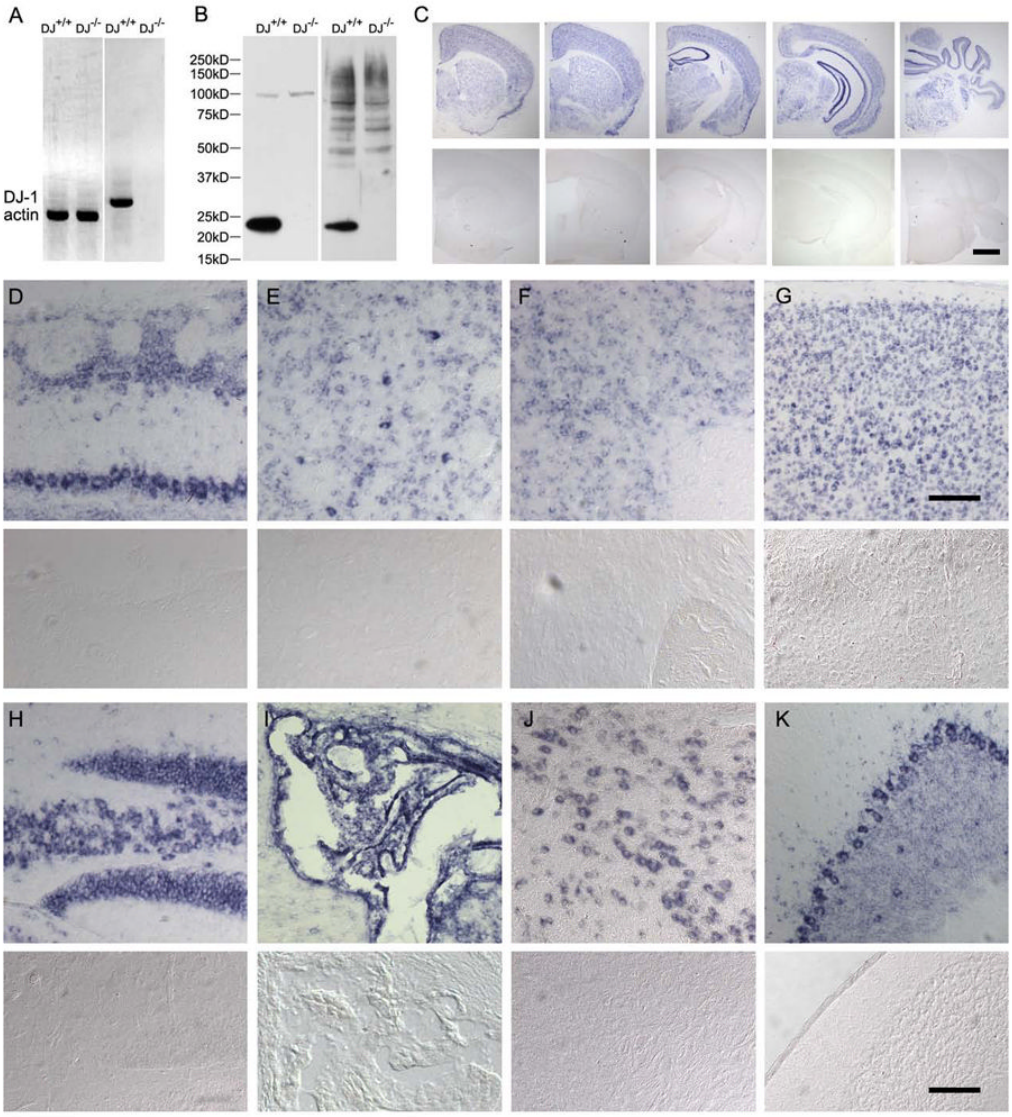

Fig. 1.

DJ-1 mRNA was ubiquitously expressed in mouse brains. A. Confirmation of lacking DJ-1 mRNA in DJ-1 deficient mouse brain by RT-PCR. B. Confirmation of lacking DJ-1 protein in DJ-1 deficient mouse brain by western blot. Left and right panels were blotted with anti-DJ-1 antibodies from Signet and Santa Cruz, respectively. C. In situ hybridization staining demonstrated widespread expression of DJ-1 mRNA in mouse brains (upper panel), while absence in DJ-1 deficient mouse (lower panel). D through K. Representative pictures showing the DJ-1 mRNA expression in brain regions including olfactory bulb (D), striatum (E), accumbens (F), cerebral cortex (G), hippocampus (H), choroid plexus and ependymal cells (I), ventral midbrain (J), cerebellum (K). Bottom part of each panel showed no DJ-1 mRNA staining in DJ-1 deficient mice in D through K. Scale bar: 1 mm (C), 100 μm (D, E, F, H, I, J, K), 200 μm (G)

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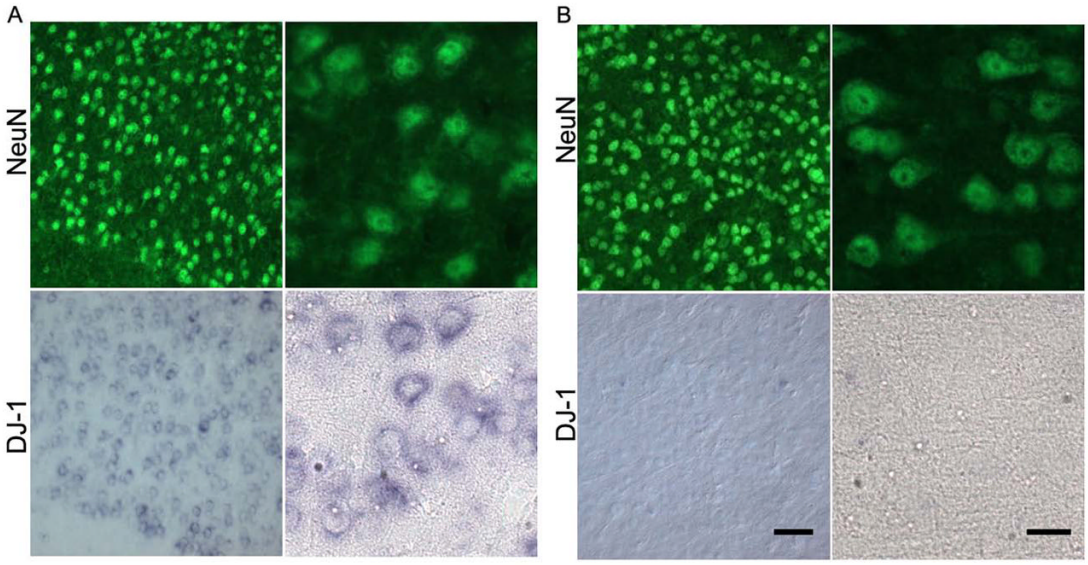

C

	$NeuN^+DJ^+$	$NeuN^+DJ^-$	Percentage of DJ-1 expression in $NeuN^+$ cell
cerebral cortex	473	18	96.3%
thalamus	809	15	98.2%
dorsal striatum	509	19	96.4%
amygdala	212	3	98.6%

Fig. 2.

DJ-1 mRNA was expressed in most neurons. Representative pictures of cerebral cortex were shown. A. Colocalization of DJ-1 mRNA (dark blue) and NeuN (green) was observed in most neurons. NeuN staining was primarily localized in the nucleus with diffused staining in the cytoplasm. The in situ hybridization staining for DJ-1 mRNA was cytoplasmic. B. Absence of DJ-1 mRNA staining in DJ-1 deficient mice. Lower and higher power images were shown in left and right panels in A, B, respectively. C. Cell counting results suggested greater than 96.3% neurons express DJ-1 mRNA in cerebral cortex, thalamus, dorsal striatum and amygdala. Scale bar: 50 μm (A, B, left panel), 20 μm (A, B, right panel)

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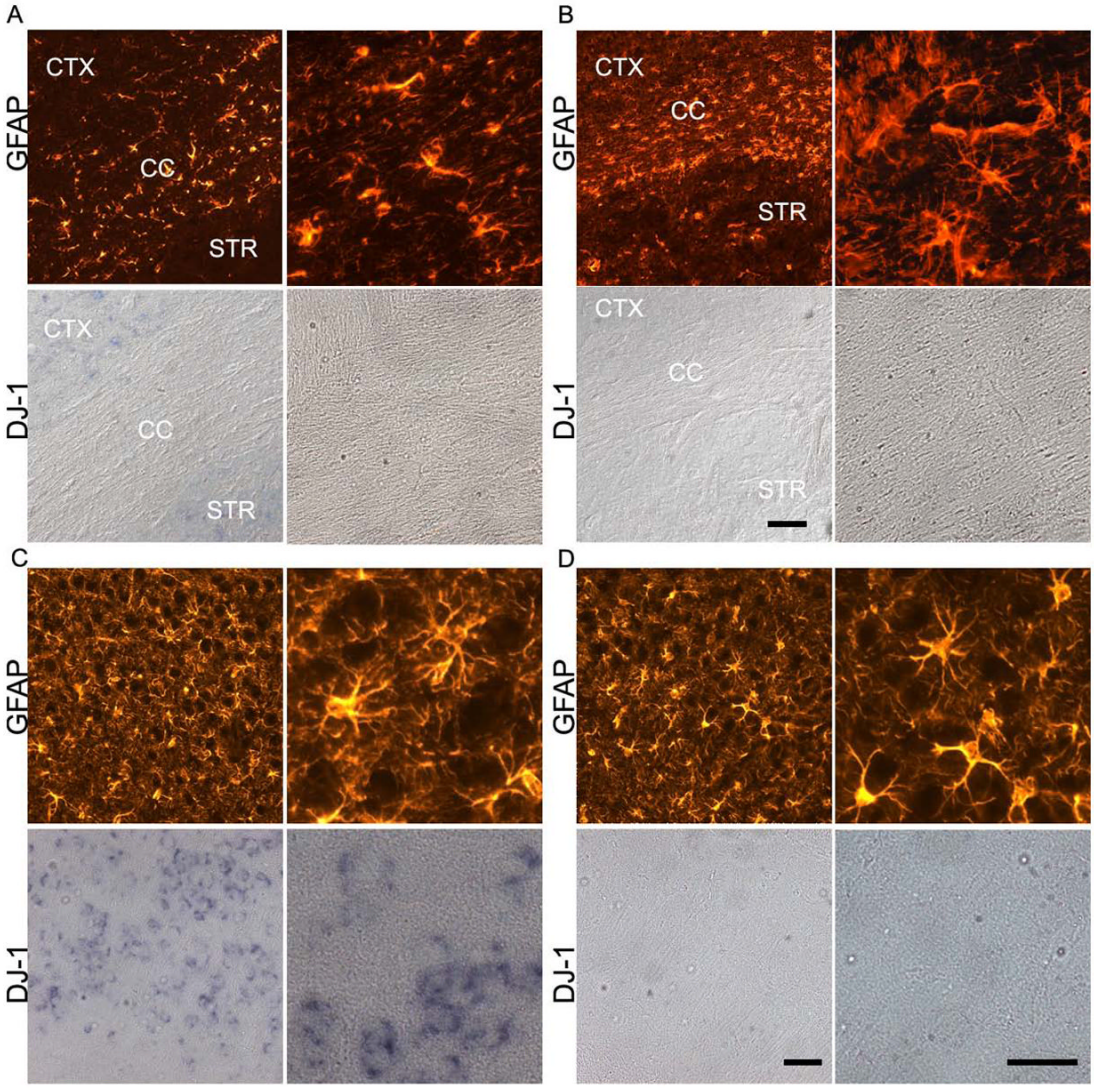

Fig. 3.

No DJ-1 mRNA was detected in astrocytes. Magnified views were shown in right panels of A, B, C and D. A. Abundant GFAP signal (red) but no DJ-1 mRNA was detected in corpus callosum (CC) in mouse brain. Note: DJ-1 positive cells (dark blue) were seen in cortex (CTX) and striatum (STR) on the same section. C. Expression of DJ-1 mRNA in astrocytes was also absent in gray matter of mouse brain. Representative pictures of cerebral cortex were shown. B and D: No DJ-1 positive cell was detected in DJ-1 deficient mice in corpus callosum (B) and other brain regions (D). Scale bar: 100 μm (A, B, left panel), 50 μm (C, D, left panel), 30 μm (A, B, C, D, right panel)

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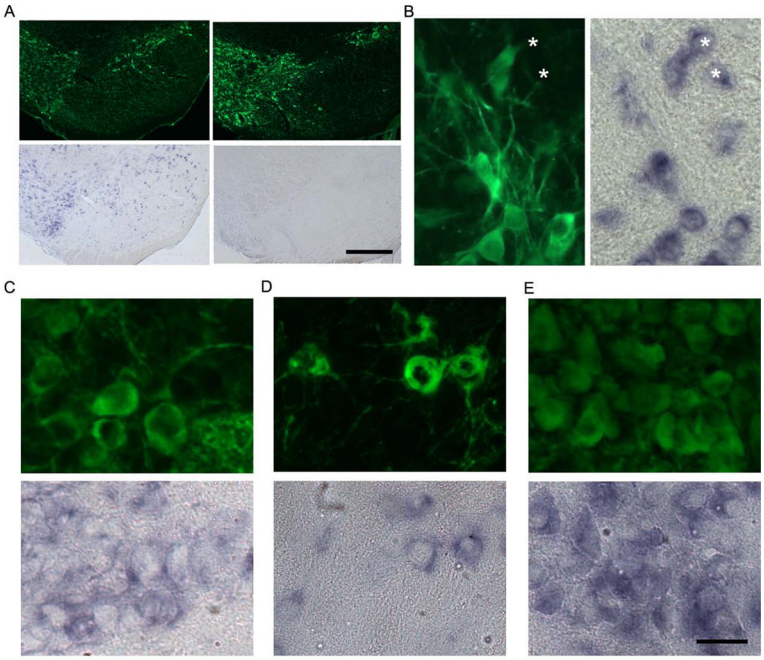

F

	TH+DJ+	TH+DJ-	Percentage of DJ-1 expression in TH+ cell
retrorubral area	63	0	100.0%
locus coeruleus	131	1	99.2%
hypothalamus	73	6	92.4%
olfactory bulb	368	19	95.1%
SNc+VTA	424	0	100.0%

Fig. 4.

DJ-1 mRNA was expressed in nearly all dopamine neurons in SNc and VTA and other dopamine neuron groups. A. Lower power images showed similar distribution pattern of TH positive neurons (green) and DJ-1 mRNA signal (dark blue) in ventral midbrain in left panel. Right panel showed no DJ-1 mRNA signal in DJ-1 deficient mice. B. Magnified view showed the colocalization between DJ-1 and TH positive cells in ventral midbrain. Asterisks indicated some DJ-1 positive cells lacking TH staining. Expression of DJ-1 mRNA was also found in most TH positive cells in olfactory bulb (C), retrorubral area (D) and locus coeruleus (E). F. Cell counting results suggested DJ-1 mRNA was expressed by all TH positive neurons in ventral midbrain and retrorubral area and by most TH positive cells in olfactory bulb, locus coeruleus and hypothalamus. Scale bar: 0.5 mm (A), 30 μm (B, C, D, E)

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Table 1

Relative expression levels of DJ-1 mRNA in mouse brain. DJ-1 mRNA intensities were rated as negative (−), weak (+), moderate (++) and strong (+++).

Brain region	Density
Telencephalon	
Glomerular layer of olfactory bulb(Gl)	++
Olfactory nerve layer(ON)	−
Mitral cell layer of olfactory bulb(Mi)	+++
Anterior olfactory nu, external(AOE)	++
Granular cell layer of olfactory bulb (GrO)	+
Piriform cortex(Pir)	++
Olfactory tubercle(Tu)	++
Accumbens nucleus(Acb)	+
Primary motor cortex(M1)	++
Secondary motor cortex(M2)	++
Primary somatosensory cortex(S1)	++
Cingulate cortex area 1–2(Cg1-2)	++
Hippocampus	
Dentate gyrus(DG)	++
Pyramidal cell layer of hip(Py)	+++
Molecular layer(Mol)	−
Lacunosum moleculare layer(LMol)	−
Fimbria of the hippocampus(Fi)	−
Subiculum(S)	++
Amygdala complex	
Anterior cortical amygdaloid nucleus(Aco)	+
Medial amygdaloid nucleus, anterodorsal(MeAD)	++
Basolateral amygdaloid nucleus, anterior(BLA)	++
Caudate putamen(Cpu)	+
Choroid plexus(chp)	++
Corpus callosum(cc)	−
Diencephalon	
Magnocellular preoptic nucleus(MCPO)	+
Thalamus	
Centromedial thalamic nucleus(CM)	+
Ventromedial thalamic nucleus(VM)	+
Ventrolateral thalamic nucleus(VL)	+
Reticular thalamic nucleus(Rt)	++
Ventral posterolateral thalamic nucleus(VPL)	++
Hypothalamus	

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Brain region	Density
Anterior hypothalamic area(AHA)	+
Ventromedial hypothalamic nucleus(VMH)	+
Periventricular nucleus(Pe)	+
Dorsomedial hypothalamic nucleus(DM)	+
Arcuate nucleus(Arc)	+
Lateral hypothalamic area(LH)	+
Supramamillary nucleus(SuM)	+
Mesencephalon	
Substantia nigra	
Lateral part(SNL)	+
Compact part(SNC)	++
Reticular part(SNR)	+
Ventral tegmental area(VTA)	++
Optic nerve layer(Op)	−
Deep mesencephalic nucleus(DpMe)	+
Nucleus of the brachium(BIC)	−
Rhombencephalon	
Cerebellum	
Granular cell layer	++
Purkinje cell layer	+++
Locus coeruleus(LC)	+++
Dorsal tegmental nucleus, central part(DTgC)	+
Motor trigeminal nucleus(Mo5)	+
Spinal trigeminal nucleus oral part(SP5O)	+