Journal of Alzheimer's Disease 23 (2011) 1–3
IOS Press

Supplementary Data

Neuronal Hemoglobin is Reduced in Alzheimer's Disease, Argyrophilic Grain Disease, Parkinson's Disease, and Dementia with Lewy Bodies

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Accepted 11 October 2010

Supplementary Table S1
Summary of cases used in the present study. FC: frontal cortex; SN: substantia nigra; Hip: hippocampus; amyg: amygdala; med oblo: medulla oblongata; M: male; F: female; PD: Parkinson disease; DLB: dementia with Lewy bodies; AD: Alzheimer disease-related pathology, stages of Braak; AGD: argyrophilic grain disease

	FC	SN	Hip	Amyg	Med oblo	Gender	Age	p-m delay	Neuropathology
1	X	X				M	41	5h55′	Control
2	X	X	X	X	X	M	56	3h50′	Control
3	X	X	X	X	X	M	39	3h30′	Control
4	X	X	X	X	X	M	59	6h25′	Control
5	X					M	61	2h45′	Control
6	X					M	67	5h	Control
7	X		X	X		F	73	7h	Control
8	X					M	75	9h	Control
9		X	X		X	F	78	3h40′	Control
10		X				F	71	8h30′	Control

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ISSN 1387-2877/11/$27.50

Supplementary Table S1
(*Continued*)

	FC	SN	Hip	Amyg	Med oblo	Gender	Age	p-m delay	Neuropathology
11		X				F	81	4h	Control
12		X	X		X	M	64	3h30′	Control
13	X					M	62	3h	Control
14	X					M	52	4h	Control
15	X					M	51	4h	Control
16	X					M	58	4h	Control
17		X	X		X	M	66	3h	Control
18		X				M	74	6h40′	Control
19		X				M	63	4h15′	Control
20		X		X	X	M	65	5h15′	Control
21		X				F	64	2h15′	Control
22		X			X	F	55	8h30′	Control
23	X					M	78	2h15′	Control
24	X					F	66	8h	Control
25	X					F	75	3h	Control
26	X	X			X	M	78	10h45′	PD
27	X	X	X			M	85	3h15′	PD
28	X	X			X	F	70	10h50′	PD
29	X	X			X	F	78	5h15′	PD
30	X	X				M	57	10h50′	PD
31	X				X	F		8h45′	PD
32	X				X	M	72	9h	PD
33	X					M	70	4h30′	PD
34	X		X		X	M	76	4h30′	PD
35		X	X			M	85	3h15′	PD
36		X	X		X	M	68	10h30′	PD
37	X	X				F	71	4h	PD
38	X	X			X	M	70	4h	PD
39	X	X			X	M	74	5h30′	PD
40	X	X				M	80	9h40′	PD
41	X	X				M	73	3h30′	PD
42		X				M	72	8h55′	PD
43		X				M	74	10h50′	PD
44	X		X	X	X	F	84	4h	DLB
45	X	X			X	M	76	9h40′	DLB
46	X					F	85	6h15′	DLB
47	X	X		X	X	M	81	7h	DLB
48		X				F	78	8h50′	DLB
49		X		X	X	M	82	1oh20′	DLB
50	X	X				M	78	8h30′	DLB
51	X	X	X		X	F	73	4h	DLB
52	X	X				F	87	6h	DLB
53	X		X			M	80	4h30′	DLB
54	X				X	M	74	6h15′	DLB
55		X				M	72	11h	DLB
56		X				F	78	6h30′	DLB
57	X		X	X		M	81	3h	AD IV
58	X		X	X		F	75	4h15′	AD V
59	X		X	X		M	78	9h30′	AD V
60	X	X	X	X	X	F	77	4h15′	AD VI
61	X	X	X	X		F	72	7h	AD VI
62	X	X	X			M	81	3h30′	AD VI
63	X					F	69	4h40′	AD VI
64	X		X		X	M	78	8h30′	AD V
65	X		X		X	F	77	6h20′	AD V
66	X		X	X	X	M	84	3h50′	AD VI
67	X	X	X		X	F	76	4h40′	AD VI
68	X		X	X		F	78	4h40′	AD IV
69			X			M	69	3h20′	AD III
70			X			M	72	2h30′	AD III
71			X			M	67	5h40′	AD III

Supplementary Table S1
(*Continued*)

	FC	SN	Hip	Amyg	Med obl	Gender	Age	p-m delay	Neuropathology
72			X			F	67	4h15′	AD II
73			X			F	72	3h15′	AD III
74	X		X	X		F	78	7h20′	AGD
75			X	X		F	76	8h30′	AGD
76	X		X			M	81	3h30′	AGD
77			X			F	68	2h50′	AGD
78			X	X		M	72	7h40′	AGD
79	X		X	X		M	68	8h20′	AGD

Supplementary Table S2
Summary of the antibodies and conditions used for immunohistochemistry, and simple and double-labeling immunofluorescence

Antibody	Species	Supplier	Dilution	Pre-treatment
Glial fibrillary acidic protein (GFAP)	Rabbit	Dako	1 : 250	
Amyloid-β	Mouse	Boehringer-Mannheim	1 : 50	Formic acid
Amyloid-β	Rabbit	Chemicon	1 : 25	Formic acid
Ubiquitin	Rabbit	Dako	1 : 200	
CD68	Mouse	Dako	1 : 100	
AT8	Mouse	Innogenetics or Pierce	1 : 50	
Phospho-tau Ser199	Rabbit	Calbiochem	1 : 100	
Phospho-tau Ser202	Rabbit	Calbiochem	1 : 100	
Phospho-tau Ser262	Rabbit	Calbiochem	1 : 100	
Phospho-tau Ser396	Rabbit	Calbiochem	1 : 100	
Phospho-tau Ser422	Rabbit	Calbiochem	1 : 100	
Phospho-tau Thr181	Rabbit	Calbiochem	1 : 250	
3Rtau	Mouse	Upstate	1 : 800	Formic acid
4Rtau	Mouse	Upstate	1 : 50	Formic acid
α-synuclein	Rabbit	Chemicon	1 : 250	Formic acid
Phospho-α-synuclein Ser129	Mouse	Wako	1 : 2,000	Formic acid
αB-crystallin	Rabbit	Novocastra	1 : 500	
Hemoglobin α	Rabbit	Santa Cruz	1 : 100	
Hemoglobin β	Rabbit	Santa Cruz	1 : 50	
Hemoglobin β	Mouse	Abcam	1 : 50	
Erythropoietin receptor	Sheep	Lifespan	1 : 100	
Neuroglobin	Mouse	Biovendor	1 : 100	

Supplementary Table S3
A semiquantitative evaluation of markedly reduced or absent hemoglobin α content in neurons with abnormal protein deposits in different regions and pathologies as revealed by the resolution provided by double-labeling immunofluorescence and confocal microscopy (see text for the method employed). FC: frontal cortex; Hip: hippocampus; Amyg: amygdala; med oblong: medulla oblongata referring neurons of the reticular formation and neurons of the motor nucleus of the vagus nerve; SN: substantia nigra referring dopaminergic neurons of the pars compacta; AD: Alzheimer's disease stages III–IV, and stages V–VI of NFT pathology following Braak nomenclature; AGD: argyrophilic grain disease; PD: Parkinson's disease; DLB: Dementia with Lewy bodies. x/x' represents the number of neurons with specific protein aggregates and the number of these neurons with markedly reduced or absent hemoglobin; n indicates the number of cases examined. bn indicates ballooned neurons. ND means not done

	FC	Hip	Amyg	Med oblong	SN
AD III–IV		61/61 ($n=4$)	42/41 ($n=4$)	ND	
AD V–VI	80/80 ($n=8$)	76/75 ($n=8$)	52/52 ($n=6$)		
			bn 18/18 ($n=5$)		
AGD		32/31 ($n=5$)	40/39 ($n=5$)	ND	ND
			bn 20/20 ($n=5$)		
PD			ND	29/23 ($n=9$)	18/13 ($n=9$)
DLB	18/12 ($n=5$)		22/17 ($n=5$)	32/26 ($n=7$)	32/25 ($n=7$)