Ishida Y, Okawa Y, Ito S, Shirokawa T and Isobe K. Age-dependent changes in dopaminergic projections from the substantia nigra pars compacta to the neostriatum. Neuroscience Letters, in press.

Ito S, Kimura K, Haneda M, Ishida Y, Sawada M , Isobe KI. Induction of Matrix metalloproteinases (MMP3, MMP12 and MMP13) expression in the microglia by amyloid-β stimulation via the PI3K/Akt pathways. Exp. Gerontology, in press.

Ito S, Sawada M ,Haneda M, Ishida Y, Isobe KI. Amyloid-beta peptides induce several chemokine mRNA expressions in the primary microglia and Ra2 cell line via the PI3K/Akt and/or ERK pathway. Neurosci Res. 2006 Nov;56(3):294-9.

Kato M, Takeda K, Kawamoto Y, Tsuzuki T, Kato Y, Ohno T, Hossain K, Iftakhar-E-Khuda I, Ohgami N, Isobe K, Takahashi M, Nakashima I. Novel Hairless RET-Transgenic Mouse Line with Melanocytic Nevi and Anagen Hair Follicles. J Invest Dermatol. 2006, 126(11):2547-50.

Nakai S, Matsunaga W, Isobe K, Shirokawa T.Age-dependent interactive changes in serotonergic and noradrenergic cortical axon terminals in F344 rats. Neurosci Res. 2006.54(3):220-229.

Nakai S, Matsunaga W, Ishida Y, Isobe K, Shirokawa T. Effects of BDNF infusion on the axon terminals of locus coeruleus neurons of aging rats. Neurosci Res. 2006;54(3):213-9.

Matsunaga W, Isobe K, Shirokawa T. Involvement of neurotrophic factors in aging of noradrenergic innervations in hippocampus and frontal cortex. Neurosci Res. 2006 54(4):313-8.

Takeda K, Kawamoto Y, Okuno Y, Kato M, Takahashi M, Suzuki H, Isobe K, Nakashima I. A PKC-mediated backup mechanism of the MXXCW motif-linked switch for initiating tyrosine kinase activities. FEBS Lett. 2006. 6; 580(3): 839-43.

Isobe K, Ito S, Haneda M, Ishida Y. Cellular and systemic defense system against age-promoting stimuli. Nagoya J Med Sci. 2006;68(1-2):9-18.

磯部健一、羽根田正隆、石田佳幸、伊藤佐知子. B細胞の分化;形質細胞への分化とストレス応答. Annual Review 免疫 2006

（２）学会発表

伊藤佐知子、羽根田正隆、石田佳幸、磯部健一　A-betaによるミクログリアからのmatrix metalloproteinases(MMPs)の産生第２９回日本基礎老化学会 2006. 6.16

ISHIDA Yoshiyuki, MIZUNO Daiki, ITO Sachiko, HANEDA Masataka, ISOBE Ken-ichi. The immunohistochemical study of B cell differentiation in the bone marrow with age. 第30回日本免疫学会総会2006.12.

厚生労働科学研究費補助金（長寿科学総合研究事業）

分担研究報告書

ヒト加齢脳におけるモノアミン関連遺伝子の発現分布と相互作用の解析

分担研究者　小阪憲司　日本福祉大学情報社会システム研究所　客員研究所員

研究要旨：ノルアドレナリン(NA)系およびセロトニン(5-HT)系の機能と維持に深く関与する脳由来神経栄養因子(brain-derived neurotrophic factor, BDNF)の遺伝子多型解析についてブレインバンクサンプルを用いて行った．その結果，アルツハイマー病およびレビー小体病患者の脳においては有意な相関性は見られなかった．現在，うつ病との関連性についての解析を準備中である．

A.　研究目的

城川らのラット加齢脳での研究から，前頭葉や海馬におけるNA線維の機能と維持にはBDNFが重要な役割を果たしていることが示唆されている（Matsunaga et al.2004, in press)．BDNFは5-HT線維の維持にも関与することが知られており，BDNFのモノアミン系への関与の可能性は高いと考えられる．一方，種々の抗うつ薬がBDNFの脳内動態に影響を及ぼすことが報告されていることから，抗うつ薬がBDNFを介してモノアミン系に作用している可能性もある．この観点より，うつ病患者の脳におけるモノアミンとBDNF関連遺伝子の解析を開始した．

B.　研究方法

ブレインサンプルの遺伝子解析

書面にて承諾を得た剖検サンプルを用いて遺伝子多型解析を行った．本年度は，BDNFの多型解析をflagment length PCR法を用いて行った．

（倫理面への配慮）

ブレインバンクに関しては,生命倫理面および個人情報管理面では,細心の注意を払っており，ヘルシンキ宣言の内容，遺伝学的検査に関するガイドライン(遺伝医学関連学会等１０学会および研究会，平成１５年８月)，ヒトゲノム・遺伝子解析研究に関する倫理指針(文部科学省，厚生労働省，経済産業省，平成１３年４月１日施行)および疫学研究に関する倫理指針（同，平成１４年７月１日施行）に準拠する.

C.　研究結果

遺伝子多型解析

アルツハイマー病およびレビー小体病患者の脳においてはBDNF遺伝子多型に有意な相関性は見られなかった.

D. 考察

種々のうつ病モデル動物の海馬領域ではBDNF遺伝子の発現が低下していること,またBDNFの低下は抗うつ薬投与で阻害されることが報告されている.うつ病患者の脳サンプルにおけるBDNF関連遺伝子発現を調べ,モノアミン系の病変との関連性についての解析を計画中である.

E.　結論

現時点で結論を導き出す事はできない.さらなる研究期間を要する.

G.　研究発表

1.　論文発表

Fujishiro H, Umegaki H, Isojima D, Akatsu H, Iguchi A, Kosaka K. Depletion of cholinergic neurons in the nucleus of the medial septum and the vertical limb of the diagonal band in dementia with Lewy bodies. Acta Neuropathol (Berl). 2006 Feb;111(2):109-14.

Akatsu H, Yamagata HD, Kawamata J, Kamino K, Takeda M, Yamamoto T, Miki T, Tooyama I, Shimohama S, Kosaka K. Variations in the BDNF Gene in Autopsy-Confirmed Alzheimer's Disease and Dementia with Lewy Bodies in Japan Dement Geriatr Cogn Disord. 2006;22(3):216-22.

Wollmer, M.A., Kapaki, E., Hersberger, M., Muntwyler, J., Brunner, F., Tsolaki, M., Akatsu, H., Kosaka, K., Michikawa, M., Molyva, D., Paraskevas, G.P., Lutjohann, D., von Eckardstein, A., Hock, C., Nitsch, R.M., Papassotiropoulos, A.
Ethnicity-dependent genetic association of ABCA2 with sporadic Alzheimer's disease.
Am J Med Genet B Neuropsychiatr Genet. 2006 Jul 5;141(5):534-6.

Isojima D, Togo T, Kosaka K, Fujishiro H, Akatsu H, Katsuse O, Iritani S, Matsumoto T, Hirayasu Y.
Vascular complications in dementia with Lewy bodies: a postmortem study
Neuropathology. 2006 ;26(4):293-7.

Mitsuda N, Yamagata HD, Zhong W, Aoto M, Akatsu H, Uekawa N, Kamino K, Taguchi K, Yamamoto T, Maruyama M, Kosaka K, Takeda M, Kondo I, Miki T.
A novel alternative splice variant of nicastrin and its implication in Alzheimer disease.
Life Sci. 2006 Apr 18;78(21):2444-8.

Satoh K, Hata M, Takahara S, Tsuzaki H, Yokota H, Akatsu H, Yamamoto T, Kosaka K, Yamada T.
A novel membrane protein, encoded by the gene covering KIAA0233, is transcriptionally induced in senile plaque-associated astrocytes.
Brain Res. 2006 Jul 17

Kimura R, Kamino K, Yamamoto M, Nuripa A, Kida T, Kazui H, Hashimoto R, Tanaka T, Kudo T, Yamagata H, Tabara Y, Miki T, Akatsu H, Kosaka K, Funakoshi E, Nishitomi K, Sakaguchi G, Kato A, Hattori H, Uema T, Takeda M.
The DYRK1A gene, encoded in chromosome 21 Down syndrome critical region, bridges between {beta}-amyloid production and tau phosphorylation in Alzheimer disease.
Hum Mol Genet. 2006 Nov 29

Yokota T, Mishra M, Akatsu H, Tani Y, Miyauchi T, Yamamoto T, Kosaka K, Nagai Y, Sawada T, Heese K.
Brain site-specific gene expression analysis in Alzheimer's disease patients. Eur J Clin Invest. 2006 Nov;36(11):820-30.

2. 学会発表

三木哲郎、山縣英久、満田憲昭、鐘望涛、青木守、赤津裕康、紙野晃人、武田雅俊、小原克彦、小阪憲司
PE-306ニカストリン遺伝子スプライス変異と確実例アルツハイマー病の関連
日本神経学会(2006.5.11-13)

赤津裕康、三室マヤ、中澤秀嘉、松川則之、山本孝之、堀映、吉田眞理、小阪憲司、橋詰良夫
Amyloid angiopathyを伴った Orthochromatic(sudanophilic) leukodystrophyの一例
第47回日本神経病理学会
(2006.5.24-26)

Hiroyasu Akatsu, Hidehisa Yamagata, Jun Kawamata, Kouzin Kamino, Masatoshi Takeda, Takayuki Yamamoto, Tetsuro Miki, Ikuo , Shun Shimohama, Kenji Kosaka
Variations in the Brain-Derived Neurotrophic Factor(BDNF) Gene in autopsy-confirmed Alzheimer's disease(AD) or Dementia with Lewy bodies(DLB) in Japan
第一回国際ブレインバンク会議
(2006.6.13-15)

Kazuki Satoh, Mitsumi Hata, Seiji Takahara, Tomoko Shimizu, Hidetoshi Tsuzaki, Hiroshi Yokota, Hiroyasu Akatsu, Takayuki Yamamoto, Kenji Kosaka and Tatsuo Yamada
Novel genes transcriptionally induced in senile-plaque associated astrocytes
第20回国際生化学・分子生物学会議/第11回アジア・オセアニア生化学者・分子生物学者連合会議/第79回日本生化学会大会、第29回日本分子生物学会年会および第59回日本細胞生物学会大会との共同開催(2006.6.18-23)

Hiroyasu Akatsu, Masahiro Umezu, Takashi Ishii, Hideyuki Suzuki, Takayuki Yamamoto, Takuya Katagiri, Katsuyosi Mizukami, Takashi Asada, Kenji Kosaka, Kazuhiro Uchida
Proteome analysis of choroid plexus in Alzheimer's disease
第20回国際生化学・分子生物学会議/第11回アジア・オセアニア生化学者・分子生物学者連合会議/第79回日本生化学会大会、第29回日本分子生物学会年会および第59回日本細胞生物学会大会との共同開催(2006.6.18-23)
(2006.7.15-20)

Hiroyasu Akatsu, Masahiro Umezu, Takashi Ishii, Hideyuki Suzuki, Takuya Katagiri, Katsuyosi Mizukami, Takashi Asada, Kenji Kosaka, Kazuhiro Uchida
Choroid plexus proteome analysis in Alzheimer disease
ICGP 6th Annual Meeting(2006.10.3-6)

18) Ikuo Tooyama, Tomoko Kato, Yoshihiro Konishi, Shun Shimohama, Teruyuki Tsuji, Hiroyasu Akatsu
The interaction of α1-chimaerin protein with β-amyloid in culture cells
ICGP 6th Annual Meeting(2006.10.3-6)

Kazuki Satoh, Mitsumi Hata, Seiji Takahara, Tomoko Shimizu, Hidetoshi Tsuzaki, Hiroshi Yokota, Hiroyasu Akatsu, Takayuki Yamamoto, Kenji Kosaka and Tatsuo Yamada
Novel genes transcriptionally induced in senile-plaque associated astrocytes
第36回米国ニューロサイエンス
(2006.10.14-18)

厚生労働科学研究費補助金（長寿科学総合研究事業）

分担研究報告書

高齢者および血管障害後のうつ病･認知症とモノアミンとの関連の解析

分担研究者　赤津裕康　福祉村病院長寿医学研究所　副所長

研究要旨

目的：脳卒中後うつ病(post-stroke depression: PSD)に着目し，PSDを認めた死後脳の生化学的分析によってモノアミン系の相互作用の解析を行う。

方法：福祉村病院に保存されている４００例を越す剖検凍結脳を用いて、

１） PSDの臨床像、うつ症状およびSSRI等の服薬状況の把握を行う。

２） 該当患者における血液・髄液でのノルアドレナリン（NA）系およびセロトニン（5-HT）解析を行う。

３） 該当患者脳の左右複数の脳部位（前頭葉，側頭葉，後頭葉）から試料を採取し，NA，5-HT，NA transporter，5-HT transporter を定量し，NA系および5-HT系の相互作用について解析する。

結果：剖検脳のデータベース化を行っており、現在、脳血管障害のあった患者の臨床像におけるうつ状態の把握を進め、症例の抽出の途中である。

考察：現時点では、考察可能な結果を得ていない。

A.　研究目的

脳卒中後うつ病(post-stroke depression: PSD)に着目し，PSDを認めた死後脳の生化学的分析によってモノアミン系の相互作用の解析を行う。

B.　研究方法

我々はPSD症例のあった剖検症例におい

て脳実質・髄液・血液サンプルを用いてNA, 5-HT, NA transporter, 5-HT transporterを定量し, NA系および5-HT系の相互作用について解析する。

（倫理面への配慮）

ヒトサンプルは病理解剖時に、遺伝子解析も含めての研究利用に供される事が明記してある書面にて遺族より承諾書をとっている。その後の検体はすべて匿名化され、全ての情報は個人情報管理室にて厳重に管理されている。またヒトサンプルを用いての研究は全て福祉村病院倫理委員会の承認を得て行われている。

C.　研究結果

剖検脳のデータベース化を行っており、現在、脳血管障害のあった患者の臨床像におけるうつ状態の把握を進め、症例の抽出を行っている。従って、一定の結果を得るためにさらなる研究期間を要している。

D.　考察

現時点では、考察可能な結果を得ていないが、今後症例の抽出が終了すれば抽出症例の解析を行い、PSD, 加齢とモノアミン系の関連性に一定の回答を出す事が可能になると考えている。

E.　結論

現時点で結論を導き出す事はできない。そのためにさらなる研究期間を要する。

G.　研究発表

1.　論文発表

1）Fujishiro H, Umegaki H, Isojima D, Akatsu H, Iguchi A, Kosaka K. Depletion of cholinergic neurons in the nucleus of the medial septum and the vertical limb of the diagonal band in dementia with Lewy bodies. Acta Neuropathol (Berl). 2006 Feb;111(2):109-14.

2）Kimura R, Kamino K, Yamamoto M, Kida T, Akatsu H, Uema T, Kobayashi T, Hattori H, Nuripa A, Nessa BN, Kazui H, Ikejiri Y, Tanaka T, Tanii H, Kudo T, Yoneda H, Yamagata H, Miki T, Takeda M. Albumin gene encoding free

fatty acid and beta-amyloid transporter is genetically associated with Alzheimer disease. Psychiatry Clin Neurosci. 2006 Apr;60 Suppl 1:S34-9.

3) Akatsu H, Yamagata HD, Kawamata J, Kamino K, Takeda M, Yamamoto T, Miki T, Tooyama I, Shimohama S, Kosaka K. Variations in the BDNF Gene in Autopsy-Confirmed Alzheimer's Disease and Dementia with Lewy Bodies in Japan. Dement Geriatr Cogn Disord. 2006;22(3):216-22.

4) Wollmer, M.A., Kapaki, E., Hersberger, M., Muntwyler, J., Brunner, F., Tsolaki, M., Akatsu, H., Kosaka, K., Michikawa, M., Molyva, D., Paraskevas, G.P., Lutjohann, D., von Eckardstein, A., Hock, C., Nitsch, R.M., Papassotiropoulos, A. Ethnicity-dependent genetic association of ABCA2 with sporadic Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet. 2006 Jul 5;141(5):534-6.

5) Heese K, Akatsu H. Alzheimer's disease - an interactive perspective. Curr Alzheimer Res. 2006 ;3(2):109-21.

6) Isojima D, Togo T, Kosaka K, Fujishiro H, Akatsu H, Katsuse O, Iritani S, Matsumoto T, Hirayasu Y. Vascular complications in dementia with Lewy bodies: a postmortem study Neuropathology. 2006 ;26(4):293-7.

7) Mitsuda N, Yamagata HD, Zhong W, Aoto M, Akatsu H, Uekawa N, Kamino K, Taguchi K, Yamamoto T, Maruyama M, Kosaka K, Takeda M, Kondo I, Miki T. A novel alternative splice variant of nicastrin and its implication in Alzheimer disease. Life Sci. 2006;78(21):2444-8.

8) Satoh K, Hata M, Takahara S, Tsuzaki H, Yokota H, Akatsu H, Yamamoto T, Kosaka K, Yamada T.

A novel membrane protein, encoded by the gene covering KIAA0233, is transcriptionally induced in senile plaque-associated astrocytes.
Brain Res. 2006 Jul 17

9) Waragai M, Wei J, Fujita M, Nakai M, Ho GJ, Masliah E, Akatsu H, Yamada T, Hashimoto M.
Increased level of DJ-1 in the cerebrospinal fluids of sporadic Parkinson's disease.
Biochem Biophys Res Commun. 2006 Jul 7;345(3):967-72.

10) Kimura R, Kamino K, Yamamoto M, Nuripa A, Kida T, Kazui H, Hashimoto R, Tanaka T, Kudo T, Yamagata H, Tabara Y, Miki T, Akatsu H, Kosaka K, Funakoshi E, Nishitomi K, Sakaguchi G, Kato A, Hattori H, Uema T, Takeda M.
The DYRK1A gene, encoded in chromosome 21 Down syndrome critical region, bridges between {beta}-amyloid production and tau phosphorylation in Alzheimer disease.
Hum Mol Genet. 2006 Nov 29

11) Yokota T, Mishra M, Akatsu H, Tani Y, Miyauchi T, Yamamoto T, Kosaka K, Nagai Y, Sawada T, Heese K.
Brain site-specific gene expression analysis in Alzheimer's disease patients. Eur J Clin Invest. 2006 Nov;36(11):820-30.

12) Makino S, Kaji R, Ando S, Tomizawa M, Yasuno K, Goto S, Matsumoto S, Tabuena MD, Maranon E, Dantes M, Lee LV, Ogasawara K, Tooyama I, Akatsu H, Nishimura M, Tamiya G.
Reduced Neuron-Specific Expression of the TAF1 Gene Is Associated with X-Linked Dystonia-Parkinsonism.
Am J Hum Genet. 2007 Mar; 80(3): 393-406.

13) Akatsu H, Ishiguro M, Ogawa N, Kanesaka T, Okada N, Yamamoto T, Campbell W, and Okada H
Plasma levels of unactivated thrombin activatable fibrinolysis inhibitor

(TAFI) are down-regulated in young adult women - Analysis of a normal Japanese population.
Micobiol and Immuno, 2007 in press

2. 学会発表

1）工藤幸司、古本祥三、岡村信行、丸山将浩、田代学、舟木善仁、石川洋介、加藤元久、赤津裕康、山本孝之、成田勉、古川勝敏、岩田錬、伊藤正敏、谷内一彦、荒井哲行
アルツハイマー病の早期診断のためPETプローブの開発
東北大学先進行医工学研究機構第2回公開シンポジウム(2006.1.24)

2）赤津裕康、松本光弘、宮本圭子、山本淑子、芦田欣也、高見正雄、小橋修
胃瘻造設患者へのタンパク質補給食品（メイプロテイン)の栄養介入による栄養改善効果
第２１回日本静脈経腸栄養学会
(2006.1.27)

3）澤田徹、岡村信行、工藤幸司、谷内一彦、赤津裕康、山本孝之
アミロイドβ蛋白の画像化によるアルツハイマー病の早期診断
痴呆を語る会(2006.2.18)

4）澤田徹、岡村信行、工藤幸司、谷内一彦、赤津裕康、山本孝之
PETによるβアミロイドイメージングの可能性
第35回日本神経放射線学会(2006.2.23)

5）赤津裕康、宮本圭子、谷水清美、山本淑子、小橋修、山本孝之
栄養療法にて肝性脳症をコントロールし得た末期肝癌例
第6回愛知NST研究会(2006.2.25)

6）澤田徹、岡村信行、工藤幸司、谷内一彦、赤津裕康、山本孝之
βアミロイドイメージング
AD研究会画像診断サブコミティ
(2006.2.4)

7）澤田徹、岡村信行、工藤幸司、谷内一彦、赤津裕康、山本孝之
アミロイドイメージング用PETプローブの開発とその臨床応用

第79回日本薬理学会年会(2006.3.10)

８）山縣英久、鐘望涛、田口敬子、名倉潤、川尻真和、秦龍二、赤津裕康、紙野晃人、武田雅俊、三木哲郎
519候補遺伝子アプローチによるアルツハイマー病関連遺伝子の探索
日本内科学会総会(2006.4.14-16)

９）山縣英久、鐘望涛、田口敬子、赤津裕康、紙野晃人、川尻真和、武田雅俊、三木哲郎
PE-305リンパ球特異的蛋白チロシンキナーゼはアルツハイマー病の新規リスク遺伝子である
日本神経学会(2006.5.11-13)

１０）三木哲郎、山縣英久、満田憲昭、鐘望涛、青木守、赤津裕康、紙野晃人、武田雅俊、小原克彦、小阪憲司
PE-306ニカストリン遺伝子スプライス変異と確実例アルツハイマー病の関連
日本神経学会(2006.5.11-13)

１１）赤津裕康、水上勝義、石井俊、山本孝之、小阪憲司、片桐拓也、内田和彦、朝田隆
アルツハイマー病患者における脈絡叢のプロテオーム解析
日本精神神経学会(2006.5.11-13)

１２）赤津裕康、三室マヤ、中澤秀嘉、松川則之、山本孝之、堀映、吉田眞理、小阪憲司、橋詰良夫
Amyloid angiopathyを伴った Orthochromatic(sudanophilic) leukodystrophyの一例
第47回日本神経病理学会
(2006.5.24-26)

１３）Hiroyasu Akatsu, Hidehisa Yamagata, Jun Kawamata, Kouzin Kamino, Masatoshi Takeda, Takayuki Yamamoto, Tetsuro Miki, Ikuo , Shun Shimohama, Kenji Kosaka
Variations in the Brain-Derived Neurotrophic Factor(BDNF) Gene in autopsy-confirmed Alzheimer's disease(AD) or Dementia with Lewy bodies(DLB) in Japan
第一回国際ブレインバンク会議
(2006.6.13-15)

１４）Kazuki Satoh, Mitsumi Hata, Seiji Takahara, Tomoko Shimizu, Hidetoshi Tsuzaki, Hiroshi Yokota, Hiroyasu Akatsu, Takayuki Yamamoto, Kenji Kosaka and Tatsuo Yamada
Novel genes transcriptionally induced in senile-plaque associated astrocytes
第20回国際生化学・分子生物学会議/第11回アジア・オセアニア生化学者・分子生物学者連合会議/第79回日本生化学会大会、第29回日本分子生物学会年会および第59回日本細胞生物学会大会との共同開催(2006.6.18-23)

１５）Hiroyasu Akatsu, Masahiro Umezu, Takashi Ishii, Hideyuki Suzuki, Takayuki Yamamoto, Takuya Katagiri, Katsuyosi Mizukami, Takashi Asada, Kenji Kosaka, Kazuhiro Uchida.
Proteome analysis of choroid plexus in Alzheimer's disease
第20回国際生化学・分子生物学会議/第11回アジア・オセアニア生化学者・分子生物学者連合会議/第79回日本生化学会大会、第29回日本分子生物学会年会および第59回日本細胞生物学会大会との共同開催(2006.6.18-23)

１６）Saori Hata, Yoichi Araki, Hiroyasu Akatsu, Katsuya Urakami, Masaki Nishimura, Tadashi Nakaya, Toshiharu Suzuki.
β-Alcα, metabolic products of Alcadein, as a novel diagnostic marker in CSF of Alzheimer's disease
国際アルツハイマー学会
(2006.7.15-20)

１７）Hiroyasu Akatsu, Masahiro Umezu, Takashi Ishii, Hideyuki Suzuki, Takuya Katagiri, Katsuyosi Mizukami, Takashi Asada, Kenji Kosaka, Kazuhiro Uchida.
Choroid plexus proteome analysis in Alzheimer disease
ICGP 6th Annual Meeting (2006.10.3-6)

１８）Ikuo Tooyama, Tomoko Kato, Yoshihiro Konishi, Shun Shimohama, Teruyuki Tsuji, Hiroyasu Akatsu
The interaction of α1-chimaerin

protein with β-amyloid in culture cells.
ICGP 6th Annual Meeting(2006.10.3-6)

１９）Nobuyuki Okamura, Yukitsuka Kudo, Shozo Furumoto, Katsutoshi Furukawa, Manabu Tashiro, Motohisa Kato, Hiroyasu Akatsu, Tohru Sawada, Kazuhiko Yanai, Hiroyuki Arai
In vivo imaging of amyloid plaques in the brain: [11C]BF-227 PET study.
ICGP 6th Annual Meeting(2006.10.3-6)

２０）Kazuki Satoh, Mitsumi Hata, Seiji Takahara, Tomoko Shimizu, Hidetoshi Tsuzaki, Hiroshi Yokota, Hiroyasu Akatsu, Takayuki Yamamoto, Kenji Kosaka and Tatsuo Yamada
Novel genes transcriptionally induced in senile-plaque associated astrocytes
第36回米国ニューロサイエンス
(2006.10.14-18)

２１）赤津裕康、三室マヤ、谷由章、山本孝之、堀映、橋詰良夫
パーキンソニズムと認知症状を伴った１剖検例　第34回臨床神経病理懇話会
(2006.11.18-19)

研究成果の刊行に関する一覧表

1. Ishida, Y., Okawa, Y., S. Ito, S., Shirokawa, T. and Isobe, K. Age-dependent changes in dopaminergic projections from the substantia nigra pars compacta to the neostriatum. Neuroscience Letters, in press.

2. Nakai, S., Matsunaga, W., Ishida, Y., Isobe, K. and Shirokawa, T. Effects of BDNF infusion on the axon terminals of locus coeruleus neurons of aging rats. Neuroscience Research, 54: 213-219, 2006.

3. Nakai, S., Matsunaga, W., Isobe, K. and Shirokawa, T. Age-dependent interactive changes in serotonergic and noradrenergic cortical axon terminals in F344 rats. Neuroscience Research, 54: 220-229, 2006.

4. Matsunaga, W., Isobe, K. and Shirokawa, T. Involvement of neurotrophic factors in aging of noradrenergic innervations in hippocampus and frontal cortex. Neuroscience Research, 54: 313-318, 2006.

+Model
NSL 23992 1–5
ARTICLE IN PRESS

Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters xxx (2007) xxx–xxx
Neuroscience Letters
www.elsevier.com/locate/neulet

Age-dependent changes in dopaminergic projections from the substantia nigra pars compacta to the neostriatum

Yoshiyuki Ishida [a,b,*], Yayoi Okawa [c], Sachiko Ito [b,d], Tetsuya Shirokawa [e], Ken-ichi Isobe [b,d]

[a] *Radioisotope Research Center, Nagoya University Graduate School of Medicine, Japan*
[b] *Department of Mechanism of Aging, National Institute for Longevity Sciences, Japan*
[c] *Department of Functioning Activation, National Institute for Longevity Sciences, Japan*
[d] *Department of Immunology, Nagoya University Graduate School of Medicine, Japan*
[e] *Department of Social and Information Sciences, Nihon Fukushi University, Japan*

Received 11 January 2007; received in revised form 13 March 2007; accepted 14 March 2007

Abstract

Age-dependent changes in dopaminergic (DA) innervation of the neostriatum (Str) were studied in male F344/N rats. Projections from the substantia nigra pars compacta (SNc) to the neostriatum were quantified using electrophysiological methods at age points from 6 to 24 months. The percentage of DA neurons activated antidromically by electrical stimulation (P-index) of Str increased between 18 and 24 months. Additionally, the percentage of DA neurons showing multiple antidromic latencies from striatal stimulation (M-index), which suggests axonal branching of individual DA neurons, increased significantly between 6 and 12 months and 6 and 24 months. These results suggest that DA neurons exhibit increased axonal branching in the aged brain.

Keywords: Aging; Substantia nigra pars compacta; Electrophysiology; F344 rat

The substantia nigra pars compacta (SNc) is one of the dopaminergic (DA) nuclei in the central nervous system. Nigral dopaminergic neurons innervate many target regions in the brain and supply the main dopaminergic input to the neostriatum (Str) [12]. The axons of DA neurons terminate mainly in the dorsolateral region of the Str [7]. DA neurons play an important role in complex motor control [18] and exhibit decreases in their function during both the normal aging process and in neurodegenerative states such as Parkinson's disease. However, it remains unclear at present how dopaminergic innervation of the neostriatum changes throughout aging. Specifically, it is unclear as to whether the deficits in dopaminergic signaling are due to pre- and/or post-synaptic changes. To investigate the age-dependent changes in the projections from SNc to Str in the rat, we used *in vivo* electrophysiological techniques to antidromically activate their axon terminal fields in the Str to determine whether or not there are changes in the excitability of nigral dopaminergic terminals during aging.

Male F344/N rats (four groups; 6, 12, 18 and 24 months of age, $n=5$ for each age group) were used. Animals were housed with food and water available ad libitum on a 12 h light/dark cycle. All animal procedures complied with the Animal Research Facilities Committee of the National Institute for Longevity Sciences. Animals were anesthetized with urethane (1.2 g/kg, i.p.). Lidocaine was applied locally to all incisions. Rectal temperature was maintained at 36.5 °C by a heating pad. The ECG and EEG were monitored continuously during these experiments. Stimulating electrodes were of the bipolar type, and consisted of two insulated stainless steel wires (diameter 200 μm, tip separation 0.5 mm). Electrical pulses for the stimulus site were 0.5 ms in duration with currents ranging from 0.1 to 5.0 mA, and the cycle of stimulation was 1.5 s. The electrodes were stereotaxically guided into the Str (A: 1.0 from bregma, L: 3.7, D: 4.0). The electrical activity of nigral dopaminergic neurons was recorded extracellularly with glass pipette microelectrodes filled with 2 M NaCl. Electrode resistance ranged from 10 to 20 MΩ. A recording electrode was inserted from a point (A: 2.1 from lambda, L: 2.0). SNc neurons were usually encountered 6.8–8.0 mm below the cortical surface. In each animal, 21–23 SNc neurons were recorded by

* Corresponding author. Tel.: +81 744 2411; fax: +81 744 2416.
E-mail address: ishida@med.nagoya-u.ac.jp (Y. Ishida).

0304-3940/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2007.03.031

Please cite this article in press as: Y. Ishida et al., Age-dependent changes in dopaminergic projections from the substantia nigra pars compacta to the neostriatum, Neurosci. Lett. (2007), doi:10.1016/j.neulet.2007.03.031

moving a recording electrode within about 200 μm rostrocaudally or mediolaterally to avoid sampling bias. The DA neurons in the SNc were identified according to well-established criteria [4,7,14]. Briefly, nigral dopaminergic neurons exhibited a characteristic wide spike duration (~2 ms), and slow spontaneous firing between 0.5 and 6 Hz. SNc neurons exhibited responses from striatal stimulation considered to be antidromic in nature provided that the following criteria were satisfied: (1) constant latency of the initial segment spike at a low stimulation frequency (1 Hz) (Fig. 1A), (2) ability to follow stimulation at high frequencies (>200 Hz) and (3) collision with spontaneous or orthodromic spikes. The stimulating current was adjusted to a value which was just sufficient to elicit an antidromic response to every stimulus. The threshold current was measured by varying the stimulating current in 0.01 mA steps. The antidromic latency was also measured for individual SNc neurons. A certain proportion of SNc neurons showed two or more discrete antidromic latencies (multiple antidromic latencies) from neostriatal stimulation. In these SNc neurons, if the stimulus current was increased to threshold or beyond, the long latency response with the low threshold often abruptly jumped to short latency responses that occured at higher intensities (Fig. 1B). We measured the threshold current for the long latency response as well as the short ones for all SNc neurons that responded antidromically. Fig. 1C demonstrates histologically (HE staining) that the recording sites and the tracts of the recording electrodes were included in the SNc.

We employed two electrophysiological measurements [1,10,13,17] to quantify the density of SNc axons: (1) the percentage of SNc neurons activated antidromically from Str (P-index: number of SNc neurons with antidromic latencies/number of recorded SNc neurons) and (2) the ratio of SNc neurons that showed two or more discrete antidromic latencies from Str at different intensities of stimulus currents (M-index: number of SNc neurons with multiple antidromic latencies/number of SNc neurons with antidromic latencies). In many systems, these multiple antidromic latencies are regarded as the activation of two or more different axonal branches. Since high frequency stimulation often leads to the blockage of impulse conduction at branch points in invertebrates, we used a frequency of stimulation low enough to avoid impulse conduction failure. In the present study, the stimulus currents (0.1–5.0 mA) always produced 100% SNc antidromic responses, with no instances of impulse conduction failure. The data are expressed as mean ± S.E., and were compared by one-way analysis of variance (one-way ANOVA) with a Bonferroni/Dunn post-hoc analysis.

The total number of SNc neurons recorded from five animals for each age group are as follows: 6 months of age (6 months), $n = 108$; 12 months, $n = 107$; 18 months, $n = 108$; 24 months, $n = 107$. To quantify a change in the density of SNc projections in Str, we first focused on the percentage of SNc neurons activated antidromically from electrical stimulation of the Str (P-index). Fig. 2A shows that the mean P-index was maintained between 6 (57.4 ± 6.0%) and 12 months of age (58.0 ± 3.1%), and then the P-index decreased slightly between 12 and 18 months of age (50.0 ± 6.2%). Unexpectedly, we observed a marked increase in the P-index at 24 months of age (71.9 ± 3.9%). The mean P-index obtained at the age of 24 months was significantly higher than that at 18 months. The utility of the M-index was based on the view that these multiple antidromic latencies resulted from the activation of two or more different axonal branches around a single stimulus locus. Therefore, the M-index provided a phys-

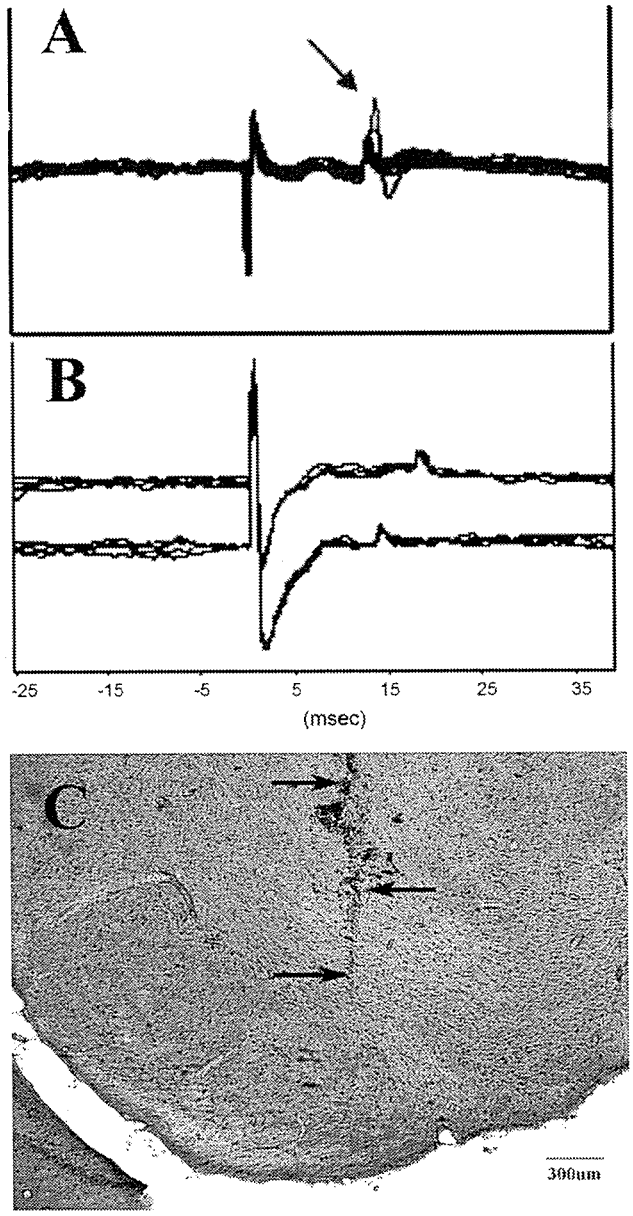

Fig. 1. Antidromic responses of DA neurons to electrical stimulation of Str. (A) At a current of 0.88 mA, an antidromic response with a single antidromic latency was evoked at a latency of 11.4 ms in a 12-month-old rat. Ten consecutive sweeps were superimposed in the noncollision trials. The majority of the antidromic action potentials consist of the initial segment spike only, but one consists of a full initial segment-somatodendritic spike (arrow). (B) The multiple antidromic response to Str stimulation of a single DA neuron in a 12-month-old rat. At a current of 1.25 mA, antidromic response was evoked at a latency of 17.4 ms (upper trace), where at a current of 3.58 mA the latency abruptly jumped to 13.6 ms (lower trace). Five consecutive sweeps were superimposed in the noncollision trials. The stimulus was applied at time 0. (C) The tracts of the recording electrodes (arrows) were included in the substantia nigra pars compacta. Scale bar, 300 μm.

Please cite this article in press as: Y. Ishida et al., Age-dependent changes in dopaminergic projections from the substantia nigra pars compacta to the neostriatum, Neurosci. Lett. (2007), doi:10.1016/j.neulet.2007.03.031

iological index of axonal branching of individual SNc neurons. Fig. 2B shows the time-course of changes in the M-index in the Str. In Str, we observed a marked increase in the average M-index between 6 (31.4 ± 0.6%) and 12 months of age (69.7 ± 7.8%). The mean M-indices obtained at the age of 12 months were significantly higher than that at 6 months. The M-index slightly leveled off at 18 months of age (48.9 ± 3.4%) and then slightly increased at the age of 24 months (58.4 ± 5.9%). The increase in the average M-index at 12 or 24 months was significantly higher than that at 6 months. These results suggested that nigral dopaminergic neurons exhibit increased axonal branching in the Str during aging.

We classified axon terminals of nigral dopaminergic neurons into three types based on their antidromic responses: (1) single-threshold terminals with single antidromic latency (single-threshold), (2) low threshold terminals with long latency, and (3) the high threshold terminals with short latency. To investigate the age-dependent changes in the physiological properties of axon terminals of individual SNc neurons, we compared the threshold–latency relationship of each terminal type in SNc. Based on our analysis of the threshold–latency relationship of DA neurons (Table 1), we concluded that the dominant terminals varied with advancing age; the single-threshold terminals were dominant until 6 months, then the dominance in threshold shifted from single to low/high after 12 months. The age-dependent changes observed in the excitability of nigral dopaminergic neuron terminals during aging suggests that there may be changes in the excitability of individual terminals during aging.

In the present study, we found age-dependent changes in the excitability of dopaminergic terminals strongly suggestive of changes in the DA innervation of Str over time. Specifically, these results suggest an increase in axonal branching between 6 and 12 months, and an increase in innervation density between 18 and 24 months. The variance seen in Fig. 2A (and also in 2B) differs among individual age groups, but we think these variances are not the result of sampling bias but rather the age-related effect on the animals. In the young animals, there was some variance which decreased at 18 months and finally increased at 24 months. We observed the same trends in our previous work [10] and the work by Suzuki's group [22]. Thus, we speculate the variability among individual age groups may be related to the age-dependent effect on the animals.

Though the P- and M-indices of DA neurons changed with age, other parameters of antidromic responses such as threshold and latency were retained during aging (Table 1). It has also been suggested that there are no significant differences in the electrophysiological parameters such as firing rate, firing rate distribution and firing pattern between 3 months and 24–28 months F344 rats [5]. In addition, the number of TH-positive neurons does not change between 3 and 6 months and 19 and 21 months in F344 rats using unbiased stereology [2]. Thus, we hypothesize that the properties of DA neurons in the SNc may be retained during aging and the age-related changes observed may be due to changes in axonal properties in target regions such as the neostriatum.

The tissue levels of DA and its metabolites in the Str over time still remain to be elucidated. Some reports showed DA and its metabolites levels were decreased in the Str of aged F344 rats [6,8,15]. Others showed no significant differences in DA and its metabolites levels in the Str between young and aged F344 rats [9,16,21]. However, a decrease in DA receptors in the Str of aged F344 rats has been consistently observed [19,22]. Thus, the sprouting of dopaminergic axon terminals in the Str might be in response to the loss of DA receptors with increasing age.

Recently, we reported age-dependent changes in the noradrenergic (NA) innervation of frontal cortex and hippocampus of F344 rats that suggested a decrease in density between 7 and 15 months, and increased axonal branching between 15 and 24 months [10,11]. Quantitative autoradiography reflecting the binding of ligands specific for noradrenergic receptor subtypes in frontal cortex and hippocampus showed no significant differences for any receptor subtype between young and aged-normal rats [3]. Only the binding of ligands for beta-1 receptors in cerebral cortex and hippocampus was likely to decrease between 5 and 24 months. These are partially consistent with our previous data. However, by using inhibitors of NA uptake and NA release in pre-synaptic terminals of LC axons, we found that the release activity mediated by the pre-synaptic autoreceptor

Table 1
The age-dependent changes in mean antidromic latency, and the number of latency jumps of DA neurons

	6 months	12 months	18 months	24 months
Mean latency (ms)				
Single	11.7 ± 1.0	13.6 ± 0.6	13.6 ± 1.0	12.5 ± 0.6
Multiple-short	12.2 ± 0.7	11.9 ± 0.5	12.5 ± 0.4	12.1 ± 0.5
Multiple-long	14.2 ± 0.8	14.4 ± 0.6	15.0 ± 0.6	15.1 ± 0.6
Mean threshold (mA)				
Single	2.24 ± 0.29	2.40 ± 0.32	2.58 ± 0.26	2.77 ± 0.43
Multiple-high	2.16 ± 0.40	2.47 ± 0.22	3.10 ± 0.30	2.75 ± 0.22
Multiple-low	1.13 ± 0.24	1.24 ± 0.17	1.62 ± 0.22	1.63 ± 0.22
Number of latency jumps	20	41	27	44

No significant difference are obtained as follows: Bonferroni/Dunn-test. single latency $F(3.16) = 1.28$, $P = 0.31$; multiple-short latency, $F(3.16) = 0.21$, $P = 0.89$; multiple-long latency, $F(3.16) = 0.47$, $P = 0.71$ single-threshold, $F(3.16) = 0.47$, $P = 0.71$; multiple-high threshold, $F(3.16) = 1.86$, $P = 0.18$; multiple-low threshold, $F(3.16) = 1.39$, $P = 0.28$.

Please cite this article in press as: Y. Ishida et al., Age-dependent changes in dopaminergic projections from the substantia nigra pars compacta to the neostriatum, Neurosci. Lett. (2007), doi:10.1016/j.neulet.2007.03.031

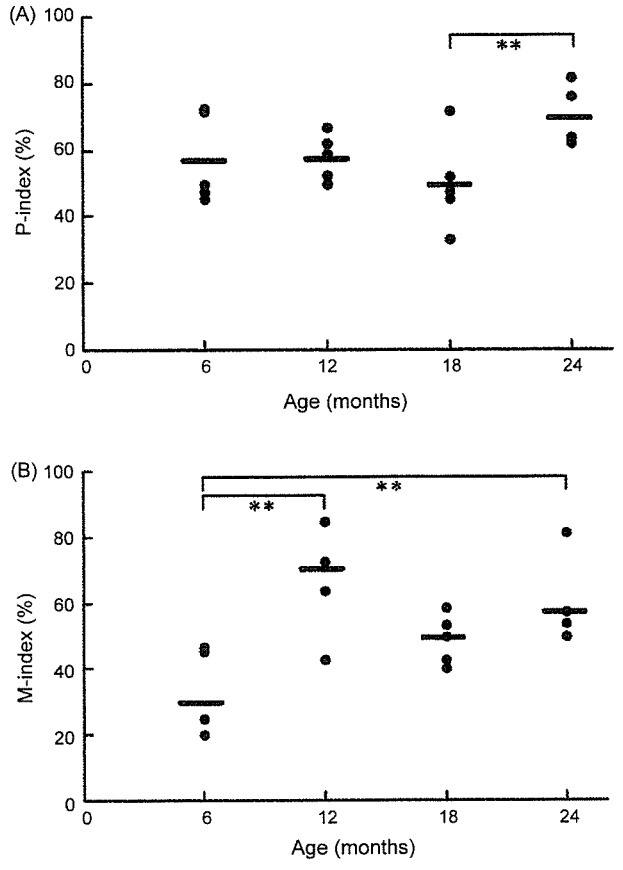

Fig. 2. (A) Age-dependent changes in P-indices (number of DA neurons with antidromic latencies/number of recorded DA neurons) in Str. Each age group consisted of five animals (filled circles). Horizontal bar indicates the mean P-index for each age group. The mean P-indices (mean ± S.D.) in Str did not change between 6 and 12 months. The mean P-indices declined gradually between 12 and 18 months. The P-indices obtained at 24 months were significantly higher than those at 18 months (Bonferroni/Dunn-test: 18 months vs. 24 months, $^{**}P<0.01$). (B) Age-dependent changes in M-indices (number of LC neurons with multiple antidromic latencies/number of DA neurons with antidromic latencies) in Str. Each age group consisted of five animals (filled circles). Horizontal bar indicates the mean M-index for each age group. The mean M-indices in Str increased significantly between 6 and 12 months, and then slightly declined at 18 months (Bonferroni/Dunn-test, 6 months vs. 12 months, $^{**}P<0.01$). The M-indices increased gradually between 18 and 24 months. Finally, the M-index increased significantly between 6 and 24 months (Bonferroni/Dunn-test, 6 months vs. 24 months, $^{**}P<0.01$).

did not change with age, but the uptake activity mediated by the NA transporter declined with age in the axon terminals of LC neurons [20]. In addition, the activity of DA transporters in the Str decreased significantly between young (6 and 12 months) and aged (18 and 24 months) F344 rats [8]. These are also approximately consistent with the timing of the axonal branching of DA neurons. Thus, we speculate that the age-related changes in the innervation of DA or NA neurons correspond most with changes in their respective transporters. Further studies are needed to examine the changes in the expression of the DA transporter in the terminal field of DA neurons during aging.

Acknowledgements

The authors thank Ms. Fulva Shah and Dr. James Tepper for technical advice and Dr. Christian Lee for comments on the manuscript. This work was supported by Research Grants for Longevity Sciences (10C-03) from the Ministry of Health and Welfare of Japan.

References

[1] G. Aston-Jones, R. Shaver, T.G. Dinan, Nucleus basalis neurons exhibit axonal branching with decreased impulse conduction velocity in rat cerebrocortex, Brain Res. 325 (1985) 271–285.
[2] K.E. Bowenkamp, L. Ujhelyi, E.J. Cline, P.C. Bickford, Effects of intrastriatal GDNF on motor coordination and striatal electrophysiology in aged F344 rats, Neurobiol. Aging 21 (1) (2000) 117–124.
[3] T.J. Collier, J.G. Greene, D.L. Felten, S.Y. Stevens, K.S. Collier, Reduced cortical noradrenergic neurotransmission is associated with increased neophbia and impaired spatial memory in aged rats, Neurobiol. 25 (2) (2004) 209–221.
[4] J.M. Deniau, C. Hammond, A. Riszk, J. Feger, Electrophysiological properties of identified output neurons of the rat substantia nigra (pars compacta and pars reticulata): evidences for the existence of branched neurons, Exp. Brain Res. 32 (1978) 409–422.
[5] A.S. Freeman, M.D. Kelland, C. Rouillard, L.A. Chiodo, Electrophysiological characteristics and pharmacological responsiveness of midbrain dopaminergic neurons of the aged rat, J. Pharmacol. Exp. Ther. 249 (3) (1989) 790–797.
[6] G.A. Gerhardt, R.E. Maloney Jr., Microdialysis studies of basal levels and stimulus-evoked overflow of dopamine and metabolites in the striatum of young and aged Fisher 344 rats, Brain Res. 816 (1999) 68–77.
[7] P.G. Guyenet, G.K. Aghajanian, Antidromic identification of dopaminergic and other output neurons of the rat substantia nigra, Brain Res. 150 (1978) 69–84.
[8] M.A. Hebert, G.A. Gerhardt, Normal and drug-induced locomotor behavior in aging comparison to evoked DA release and tissue content in fischer 344 rats, Brain Res. 797 (1998) 42–54.
[9] I.D. Hirschhorn, M.H. Makman, N.S. Sharpless, Dopamine receptor sensitivity following nigrostriatal lesion in the aged rat, Brain Res. 234 (2) (1982) 357–368.
[10] Y. Ishida, T. Shirokawa, O. Miyaishi, Y. Komatsu, K. Isobe, Age-dependent changes in projections from locus coeruleus to hippocampus dentate gyrus and frontal cortex, Eur. J. Neurosci. 12 (4) (2000) 1263–1270.
[11] Y. Ishida, T. Shirokawa, Y. Komatsu, K. Isobe, Changes in cortical noradrenergic axon terminals of locus coeruleus neurons in aged F344 rats, Neurosci. Lett. 307 (3) (2001) 197–199.
[12] S.T. Kitai, M. Sugimori, J.D. Kocsis, Excitatory nature of dopamine in the nigro-caudate pathway, Exp. Brain Res. 21 (1976) 351–363.
[13] H. Klemfuss, S.J. Young, P.M. Groves, Do antidromic latency jumps indicate axonal branching in nigrostriatal and hypothalamo-neurohypophysial neurons? Brain Res. 409 (1987) 197–203.
[14] C.R. Lee, E.D. Abercrombie, J.M. Tepper, Pallidal control of substantia nigra dopaminergic neuron firing pattern and its relation to extracellular neostriatal dopamine levels, Neuroscience 129 (2) (2004) 481–489.
[15] J.F. Marshall, A.J. Rosenstein, Age-related decline in rat striatal dopamine metabolism is regionally homogeneous, Neurobiol. Aging 11 (1990) 131–137.
[16] H.H. McIntosh, T.C. Westfall, Influence of aging on catecholamine levels, accumulation, and release in F-344 rats, Neurobiol. Aging 8 (3) (1987) 233–239.
[17] S. Nakamura, T. Sakaguchi, A. Aoki, Electrophysiological evidence for terminal sprouting of locus coeruleus neurons following repeated mild stress, Neurosci. Lett. 100 (1989) 147–152.

Please cite this article in press as: Y. Ishida et al., Age-dependent changes in dopaminergic projections from the substantia nigra pars compacta to the neostriatum, Neurosci. Lett. (2007), doi:10.1016/j.neulet.2007.03.031