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H.知的所有権の取得状況（予定を含む）

1.特許取得

「タンパク質重合体の重合核となりうるタンパク質又はその重合体が導入された細胞及びその製造法」

フランス：1964918 特許査定日：2011/10/05

英国　　：1964918 特許査定日：2011/10/05

ドイツ　：1964918 特許査定日：2011/10/05

2.実用新案登録　特になし

3.その他　特になし

厚生労働科学研究費補助金（創薬基盤推進研究事業）
認知症疾患モデル「TDP-43 脳脊髄異常蓄積マウス」の開発班
分担研究報告書

前頭側頭葉変性症モデル培養細胞 マウスの作製

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長谷川成人[3)]，野中隆[3)]，多屋長治[4)]
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[3)] 東京都医学総合研究所 病態細胞生物研究室
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研究要旨

タウ陰性前頭側頭葉変性症(FTLD)の多くは TDP-43 異常蓄積にもとづき発病するが(FTLD-TDP)，家族性 FTLD-TDP の原因遺伝子として同定されているのはプログラニュリン遺伝子 GRN である．FTLD-TDP の病態を再現するモデルを作製するため，TDP-43 トランスジェニック(Tg)マウスの解析，GRN ノックアウト(KO)マウスの系統導入と維持，TDP-43 培養細胞モデルの作製，TDP-43-Tg マウスと GRN-KO マウスの交配による TDP-43-Tg/GRN(+/-)マウスの作製と解析を行った．また，TDP-43 過剰発現培養細胞モデルにおいて TDP-43 凝集体形成の抑制作用を示した methylene blue の in vivo における効果について，（現時点では TDP-43-Tg マウスの TDP-43 異常蓄積は薬剤の効果判定に用いられるほど大量には生じないため）すでにモデルとして確立されている tau-Tg マウスを用いて調べた．さらに TDP-43 モデル開発につながる可能性を考慮し，TDP-43 と同じ RNA 結合蛋白で，その遺伝子変異が家族性筋萎縮性側索硬化症(ALS)の原因となり，大脳への異常蓄積が FTLD を引き起こすなど多くの点で TDP-43 と共通する FUS について，ALS/FTLD 圏の疾患脳脊髄における病理学的解析を行った．

A.研究目的

前頭側頭葉変性症（frontotemporal lobar degeneration: FTLD）は，脳に異常蓄積する主要蛋白質により FTLD-tau，FTLD-TDP，FTLD-FUS の 3 疾患に大別される．TDP-43 は細胞の核に局在する不均一核リボ蛋白質（hnRNP）の一種で，tau 陰性 FTLD や筋萎縮性側索硬化症（amyotrophic lateral sclerosis: ALS）の脳脊髄に異常蓄積するとともに，その遺伝子異常は家族性 ALS の原因となる．TDP-43 は主として神経細胞の細胞質に異常蓄積するが，オリゴデンドロサイトへの蓄積も認められる．TDP-43 遺伝子変異による ALS でも多数例で大脳に病変が拡がることが知られている．一方，家族性に発病する FTLD は従来 FTDP-17 と呼ばれていたが，第 17 番染色体上にある tau 遺伝子の変異により生じる FTLD-tau と，同じく第 17 番染色体上のプログラニュリン遺伝子（GRN）の変異により生じる FTLD-TDP の 2 疾患があり，今日では前者を FTDP-17 と呼ぶことが多い．GRN 変異は結果的に正常な GRN mRNA の減少につながり，病理機序としてプログラニュリンの産生低下が示唆されている．TDP-43 はアルツハイマー病やレヴィー小体型認知症患者の 3 分の 1～半数において大脳への蓄積が認められるほか，tau 異

常蓄積疾患（tauopathy）と呼ばれる疾患の多くに（全例ではないにせよ）蓄積することが見出されている．またTDP-43と同じhnRNPのひとつであるFUS（fused in sarcoma）は，タウ・TDP-43陰性FTLDにおける異常蓄積蛋白質であるとともに，やはり家族性ALSの原因遺伝子のひとつである．以上のような背景にもとづき，FTLD-TDP の病態を再現するモデルを作製し，創薬の基盤となる薬剤候補化合物のスクリーニング系を確立するのが本研究の目的である．

B.研究方法

まず分担研究者である長谷川研究員らが作出した複数系統のTDP-43 トランスジェニック(Tg)マウスの解析と，東京大学大学院農学生命科学研究科獣医生理学教室西原真杉教授が開発したGRN ノックアウト(KO)マウスの（理化学研究所からの）導入と系統維持，解析を行った．さらに，複数の異なる家族性ALSのTDP-43変異や，培養細胞で得られた情報をもとに作製した改変TDP-43遺伝子を導入したTgマウスを作製した．それらと並行して，（神経系細胞によるTDP-43 異常蓄積培養細胞モデルは既に創薬に用いることができる水準のものを作製済みなので）ヒト疾患脳脊髄に見られるTDP-43蓄積オリゴデンドロサイトの培養細胞モデルも作製した．そしてこれらのマウス脳脊髄・培養細胞について，免疫組織化学染色による観察と，界面活性剤による段階的分画～immunoblotによる解析を行った．また，これらin vitro，in vivoの実験の基盤となる情報を得るためにヒト疾患の剖検脳におけるTDP-43および，TDP-43と同じhnRNPであるFUSの病態について，生化学的，免疫組織化学的な解析を行った．

本研究の後半では，TDP-43-Tg マウスにGRN-KO マウスを交配させることによりプログラニュリン産生を抑制したTDP-43-Tgマウスを作り，TDP-43 病変の増加が生じるかどうかを検討した．また，ヒト疾患では高頻度にタウとTDP-43が同一症例に蓄積すること，両者の病態に多くの共通性が見られることから，TDP-43蓄積培養細胞モデルでスクリーニングされた治療薬候補化合物の効果を，まず（既に確立されたモデルである）tau-Tgマウスを用いて調べた．Tau-Tgマウスモデルには，ヒトP301L変異tauを導入したJNPN3系tau-Tgを Taconic 社より（他系統マウスとの交配許諾を含めて）購入し使用した．この時の候補化合物としては，既にヒトへの投与実績（マラリア，ヘモクロマトーシスなど）がある methylene blue(MB)を用いた．

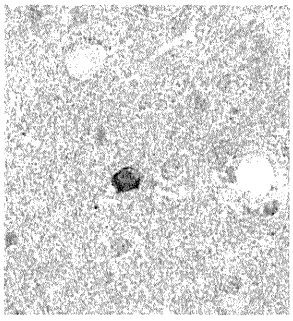
（倫理面への配慮）

Tgマウスの作製および動物実験は，当初，東京都臨床医学総合研究所および東京都精神医学研究所の実験動物倫理委員会に研究計画を提出して審査承認をうけ，その指針に従って開始した．これらの倫理委員会の審査結果はその後の改組に伴い，東京都医学総合研究所に引き継がれ承認された．また，ヒト剖検脳の解析にあたっては，ヒトゲノム・遺伝子解析研究に関する倫理基準に準じることとし，剖検時に遺族の承諾を得た場合のみ剖検材料を研究に使用した．同倫理指針策定以前に剖検になった症例については，連結可能匿名化による個人情報の保護をはかった上で使用した．ヒト剖検脳解析研究の計画も，当初，東京都臨床医学総合研究所倫理委員会の審査承認を受け，改組後は東京都医学総合研究所に引き継がれ承認された．

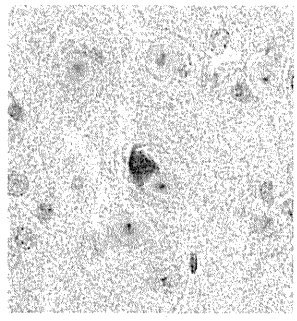
C.研究結果

①本研究開始時点ですでに作出に成功していたTDP-43-Tgの2系統（G298SおよびM337V）のうち，G298S系統では生後早期から後肢の不全麻痺が出現し，以後あまり進行せずに加齢するのに対して，M337V系統は明らかな症状は出現しない．しかし，両系統とも加齢後に（約12ヶ月齢）病理組織学的に検索すると，抗リン酸化TDP-43抗体（pS403/404およびp409/410），抗

ユビキチン抗体で陽性に染色される細胞質封入体が，間脳，脳幹と脊髄にごく少数認められた（図）．



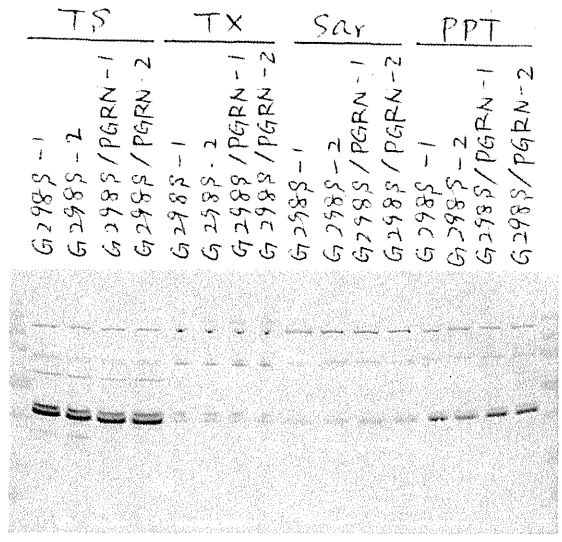
(TDP-43 pS403/404)

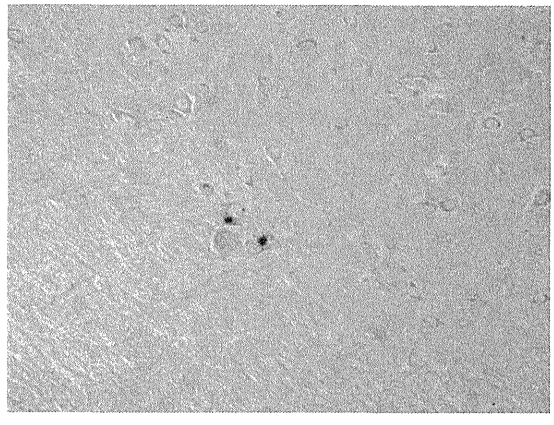


(TDP-43 pS409/410)

しかし，ヒトALS，FTLDの病理所見に比べて封入体はきわめて稀であり，おそらく量的な問題から，生化学的には異常リン酸化TDP-43の検出はできなかった．したがって，このTgマウスはヒト疾患の病態を十分再現しているとは考えられなかった．

②GRN-KOマウスはそれ自体の中枢神経系の変化は軽度で，間脳や脊髄にp62/ユビキチン陽性構造が出現するものの，ヒトのプログラニュリン変異例のようにFTLD病変が認められるわけではない．そこで，G298S，M337Vの2系統のTDP-43-Tgと，GRN-KOマウスとを交配させ，TDP-43(G298S)-Tg/GRN(+/-)マウスとTDP-43(M337V)-Tg/GRN(+/-)マウスを得た．これらの系統を約10ヶ月齢になるまで加齢させ，脳を取り出して免疫組織化学染色とimmunoblotによる解析を行ったところ，免疫組織化学染色では僅かに異常TDP-43陽性細胞の頻度が増加したように見受けられた．しかしその変化は軽微で，異常TDP-43蓄積はやはり生化学的に検出できるレベルには達しなかった．脳のTris可溶性，Tris不溶性・Tx-100可溶性，Tx-100不溶性・Sarkosyl可溶性，Sarkosyl不溶性(PPT)の4分画のimmunoblotの結果と，抗リン酸化TDP-43の免疫組織染色の結果を図に示す．





G298SやM337V単独のTgマウス脊髄横断面標本であれば，数枚の切片につき1個の封入体が観察されるのみであるこので，それと比較すれば，GRN-KOにより病変が加速されたとも考えられるが，ヒト疾患に比べればはるかに少なく，個体差を考慮に入れた場合，ただちに創薬に利用するのは難しいと判断された．

③神経系培養細胞モデルは既に良いものができているので，オリゴデンドログリア系培養細胞モデルの作製を行った．KG-1-C細胞株を用い，神経系培養細胞のモデル作製において良い結果を得ているGFPタグ付*TARDBP*ΔNLSおよび*TARDBP*(219-414)遺伝子を導入したところ，導入効率は神経系細胞SH-SY-5Yに及ばないが，異常リン酸化したTDP-43の細胞質への蓄積が生じた．遺伝子導入後にマイトマイシン-C（MMC）処理による分裂増殖停止を行い，1週

間まで培養観察が可能であった．分裂を伴わない培養細胞モデルとして薬剤投与実験に使用できると期待されるが，多数の化合物を対象としたスクリーニングを行うためには，遺伝子導入効率の向上をはかる必要がある．

④JNPN3 系 tau-Tg(P301L)マウスモデルへの MB 投与実験では，ヘテロ雌を 3 群（MB 1mg/kg/day, 0.3mg/kg/day，脱イオン水のみ）に分けて 5 ヶ月間の長期経口投与実験を行った．この系統のマウスは加齢とともに脊髄・脳幹から，間脳，大脳皮質へと tau の異常蓄積が拡がるが，マウス個体間の差が大きい．そこで，本研究では各群 10 匹以上を用い，immunoblot の結果を定量評価した．その結果，MB 1mg/kg/day 投与群では対照群に比較して異常リン酸化 tau の蓄積が有意に減少していた．

⑤FUS 免疫組織化学染色に際してホルマリン固定による染色性の低下が著しい．そこで短時間固定の剖検脳標本を用い高感度免疫組織化学染色を行って，脳における FUS の局在を再検討した．その結果，FUS は細胞核に加えニューロピルにも多数の顆粒状構造として局在していた．二重染色および（マウス脳の）immunoblot 解析の結果，ニューロピルの FUS はかなりの部分がシナプスに存在することが明らかになった．ニューロピルの顆粒状 FUS は正常脳に比べ FTLD-TDP で増加しており，（1 例のみの検索であったが）FTLD-FUS では特に増加が著しかった．

D.考察

TDP-43-Tg マウスは（野生型，変異型にかかわりなく）ヒト TDP-43 遺伝子を常法通り過剰発現させただけでは，ヒト疾患の病変（TDP-43 異常蓄積）を，治療薬開発に用いることができる（すなわち個体間で量的比較ができる）レベルで再現することはできないことが明らかになった．これは培養細胞モデルにおいて全長 TDP-43 を発現させても（ALS 変異の有無にかかわりなく）異常リン酸化などの病態再現ができないことと対応した所見であるかも知れない．TDP-43-Tg マウスが生後すぐから示す後肢麻痺は発生の段階で過剰発現した TDP-43（マウスとヒトの TDP-43 は機能的に交叉する．また，そのような表現型を示す Tg マウスでは正常の数倍のヒト TDP-43 遺伝子発現が生じている）の影響と考えられ，ALS の病態とは結びつかないと推測される．ただ，これらのマウスが高齢になると，少数ではあるが免疫組織化学染色によって異常リン酸化した TDP-43 の蓄積が神経細胞の細胞質に認められるようになることは確認された．

そこで，本研究では次に，ヒトで家族性 FTLD-TDP の原因となるプログラニュリンの低下を，遺伝的に（GRN(+/-)として）TDP-43-Tg に負荷したが，そのことによる TDP-43 異常蓄積の明瞭な増加は確認できなかった．TDP-43 異常蓄積マウスモデルの作製には，より異なる手法（発現コントロールによる生育後の過剰発現やノックインなど）を試みる必要があると考えられる．

ヒト疾患脳脊髄では神経細胞に加えオリゴデンドログリアへの TDP-43 蓄積が認められる．これは，神経細胞が分裂増殖をしない細胞であるの対してオリゴデンドログリアが常に増殖して入れ替わっている細胞種であることを考慮に入れると，病理組織標本で観察される頻度から得られる印象よりも重大な所見である．TDP-43 を標的とした抗認知症薬の創薬には，神経系細胞のみでなく，オリゴデンドログリア系細胞モデルを用いたスクリーニングも必須であると考えられる．本研究において，オリゴデンドログリア系細胞に TDP-43 異常蓄積を起こすことに成功したが，この培養細胞モデルを大量の薬剤候補化合物のスクリーニングに用いるには，遺伝子導入効率等の点でさらなる加療が必要と思われた．

ヒト疾患では tau の異常蓄積と TDP-43 の異常蓄積は多くの疾患において同一個体に共存する．

また異常蓄積したtauとTDP-43に生じる翻訳後修飾には共通点が多い．したがってひとつの薬剤がtauとTDP-43両者の凝集蓄積を抑制する可能性がある．実際，本研究で用いたMBはもともと試験管内のtau線維化を抑制する化合物として知られており，それが我々のTDP-43凝集蓄積培養細胞モデルにおいても有効性を示すことが明らかになったものである．MBでのマウスモデルでの投与研究はこれまで行われておらず，また上述のようにTDP-43-Tgマウスモデルの開発が不十分な状況から，本研究では既にモデル性が確立されたtau-Tgマウスを用いてin vivoにおける有効性の検証を行った．MBの経口投与はtauの凝集蓄積を完全に抑制することはできなかったが，統計学的に有意な凝集形成抑制作用を示し，我々の仮説を支持する結果となった．今後の創薬研究において，このような有効性の“交叉”は治療薬開発の効率を上げるために十分考慮する必要がある．

tauやαsynucleinでは異常蛋白凝集のprion様伝播が病変形成機序として注目されている.TDP-43やFUSもそれぞれC末側，N末側にprion様ドメインを持ち，異常蓄積形成の過程で同様の機序が働いている可能性がある．ただ，TDP-43やFUSは主として細胞核に局在するため，神経突起やシナプスに豊富に存在するtauやαsynucleinと異なり，神経線維連絡～軸索輸送を介した病変の拡がりが生じうるかどうかは疑問であった．しかし，本研究により，少なくともFUSは神経細胞質～神経突起にも局在し，それが疾患脳において増加することが明らかになったことから，これらRNA結合蛋白質も異常蓄積においても伝播仮説を適用できる可能性が示された．

E.結論

創薬に使用しうるレベルの高度なTDP-43異常蓄積を生じるTDP-43-Tgマウスの開発は，常法による変異遺伝子の導入や，そのマウスへの家族性FTLD-TDPの原因であるプログラニュリン低下の負荷だけでは難しいことが明らかになった．導入遺伝子の過剰発現時期の制御やノックインなどの手法を組み合わせた開発継続が必要であると考えられる．一方，培養細胞モデルは神経系細胞，オリゴデンドログリア系細胞ともほぼ実用レベルのものを利用可能であり，たとえば既存薬のスクリーニング等を行うことで培養細胞モデルから一気にヒトでの治験に移行できれば，現状でも治療薬開発は可能かも知れない．さらに本研究では，tauとTDP-43の異常蓄積機序に何らかの共通部分があり，ひとつの薬剤が双方に有効性を示す可能性があることが確認された．これは，TDP-43-Tgマウスモデル開発が進捗していない状況に置ける治療薬開発研究において考慮に値する知見であると思われる．

F.健康危険情報

特になし

G.研究発表

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H.知的所有権の取得状況（予定を含む）

1.特許取得　特になし

2.実用新案登録　特になし

3.その他　特になし

【研究成果の刊行(添付)に関する一覧表】

原著

著者名	論文タイトル名	雑誌名	巻・号	ページ	出版年
Masuda-Suzukake M, Nonaka T, Hosokawa M, Oikawa T, Arai T, Akiyama H, Mann DMA, Hasegawa M	Prion-like spreading of pathological α-synuclein in brain	Brain	136	1128-1138	2013
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総説

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秋山治彦	認知症疾患モデル「TDP-43 脳脊髄異常蓄積マウス」の開発	Annual Review 神経 2013		75-80	2013

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Prion-like spreading of pathological α-synuclein in brain

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α-Synuclein is the major component of filamentous inclusions that constitute the defining characteristic of neurodegenerative α-synucleinopathies. However, the molecular mechanisms underlying α-synuclein accumulation and spread are unclear. Here we show that intracerebral injections of sarkosyl-insoluble α-synuclein from brains of patients with dementia with Lewy bodies induced hyperphosphorylated α-synuclein pathology in wild-type mice. Furthermore, injection of fibrils of recombinant human and mouse α-synuclein efficiently induced similar α-synuclein pathologies in wild-type mice. C57BL/6J mice injected with α-synuclein fibrils developed abundant Lewy body/Lewy neurite-like pathology, whereas mice injected with soluble α-synuclein did not. Immunoblot analysis demonstrated that endogenous mouse α-synuclein started to accumulate 3 months after inoculation, while injected human α-synuclein fibrils disappeared in about a week. These results indicate that α-synuclein fibrils have prion-like properties and inoculation into wild-type brain induces α-synuclein pathology *in vivo*. This is a new mouse model of sporadic α-synucleinopathy and should be useful for elucidating progression mechanisms and evaluating disease-modifying therapy.

Keywords: α-synuclein; Lewy bodies; Parkinson's disease; propagation

Introduction

Filamentous inclusions composed of α-synuclein in nerve cells or glial cells are the defining neuropathological feature of a group of neurodegenerative diseases including Parkinson's disease, dementia with Lewy bodies, and multiple-system atrophy (Goedert, 2001). In these so-called α-synucleinopathies, α-synuclein is deposited in a hyperphosphorylated form with β-sheet-rich, fibrillar structure (Spillantini *et al.*, 1997, 1998; Baba *et al.*, 1998; Wakabayashi *et al.*, 1998; Fujiwara *et al.*, 2002). Missense mutations (A30P, E46K and A53T) in the α-synuclein gene (Polymeropoulos *et al.*, 1997; Kruger *et al.*, 1998; Zarranz *et al.*, 2004) and multiplications of the region (Singleton *et al.*, 2003; Chartier-Harlin *et al.*, 2004; Ibanez *et al.*, 2004,) have been identified in familial forms of Parkinson's disease and dementia with Lewy bodies, indicating that abnormalities of α-synuclein cause

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these diseases. Neuropathologically, α-synuclein lesions are believed to spread progressively throughout the brain and their spread correlates to the staging of clinical symptoms (Muller *et al.*, 2005), as in the case of tau pathology in Alzheimer's disease (Braak and Braak, 1991). Kordower *et al.* (2008) and Li *et al.* (2008) reported that embryonic neurons transplanted into the striatum of an individual with Parkinson's disease developed Lewy body-like pathologies, suggesting that pathological α-synuclein may be transmissible from diseased neurons to healthy neurons. Recent studies have also shown that exogenous α-synuclein fibrils induced Lewy body pathology in cultured neurons (Desplats *et al.*, 2009; Emmanouilidou *et al.*, 2010; Nonaka *et al.*, 2010; Volpicelli-Daley *et al.*, 2011), transgenic mouse brain (Mougenot *et al.*, 2012; Luk *et al.*, 2012*b*) and wild-type mouse brain (Luk *et al.*, 2012*a*). In addition, a growing body of evidence indicates that self-propagating protein aggregates play central roles in many neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease (Clavaguera *et al.*, 2009; Mougenot *et al.*, 2012; Luk *et al.*, 2012*b*; Stohr *et al.*, 2012). In this work, we have tested whether inoculation of insoluble α-synuclein from brains with dementia with Lewy bodies and synthetic mouse and human α-synuclein fibrils can induce α-synuclein pathology in wild-type mice. As a result, we have established a new mouse model of sporadic α-synucleinopathy using wild-type mice.

Materials and methods

Preparation of recombinant α-synuclein monomer and fibrils

Human and mouse α-synuclein were expressed in *E. coli* BL21 (DE3) cells, as described (Masuda *et al.*, 2006*b*). To avoid the production of α-synuclein dimers induced by misexpression of cysteine-containing α-synuclein, the Y136-TAT construct was used (Masuda *et al.*, 2006*a*). α-Synuclein was purified by boiling, Q-Sepharose® ion exchange chromatography and ammonium sulphate precipitation, before dialysis against 30 mM Tris–HCl, pH 7.5. Recombinant proteins were centrifuged at 113 000*g* for 20 min at 4°C to remove insoluble materials and used as α-synuclein monomer. Protein concentrations were determined as described (Yonetani *et al.*, 2009). Purified human and mouse α-synuclein (7 mg/ml) were incubated at 37°C in a shaking incubator (200 rpm) in 30 mM Tris–HCl, pH 7.5, containing 0.1% NaN_3, for 72 h. α-Synuclein fibrils were pelleted by spinning the assembly mixtures at 113 000*g* for 20 min.

Preparation of the insoluble fraction of dementia with Lewy bodies brain

Fresh frozen brain tissue from a patient with dementia with Lewy bodies (phosphorylated α-synuclein pathology is shown in Supplementary Fig. 9) was homogenized in 18 volumes (w/v) of Buffer A (10 mM Tris-HCl, pH 7.4, 0.8 M NaCl, 1 mM EGTA, and 10% sucrose), and sarkosyl was added to the homogenate at a concentration of 2%. The mixture was incubated for 30 min at 37°C, sonicated and spun at 9100*g* for 10 min at 25°C. The supernatant was further centrifuged at 113 000*g* for 20 min at 25°C, and the sarkosyl-insoluble pellet was washed with Buffer A. The pellet was taken up in saline, sonicated and centrifuged at 800*g* for 5 min. The supernatant was used for stereotaxic injection.

Stereotaxic surgery

Four- to six-month-old female C57BL/6J mice (CLEA Japan, Inc.) anaesthetized with 50 mg/kg pentobarbital sodium were injected with 10 μg of recombinant α-synuclein monomer, fibrils or 5 μl of insoluble fraction of dementia with Lewy bodies brain into substantia nigra (anterior-posterior: −3.0 mm; medial-lateral: −1.3 mm; dorsal-ventral: −4.7 mm from the bregma and dura) using a 10-μl Hamilton syringe. Mice were anaesthetized with isoflurane and killed by decapitation. For immunohistochemistry, brains were fixed in 10% formalin neutral buffer solution (Wako), and for biochemical analysis, brains were snap-frozen on dry ice and stored at −80°C. All experimental protocols were approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Medical Science.

Immunohistochemistry

Brains fixed in 10% formalin were cut on a vibratome (Leica) at 50 μm thickness. The free-floating sections were treated with 0.5% H_2O_2 in methanol for 30 min to inactivate peroxidase and blocked with 10% calf serum in PBS. Sections were immunostained with appropriate antibodies. Antibodies used in this study are summarized in Supplementary Table 1. After incubation with the biotinylated-secondary antibody (Vector), labelling was detected using the ABC staining kit (Vector).

Confocal microscopy

For double-label immunofluorescence for phosphorylated α-synuclein and ubiquitin or phosphorylated α-synuclein and p62, brain sections were incubated overnight at 4°C in a cocktail of 1175 and anti-ubiquitin or anti-p62 antibody. The sections were then washed and incubated in a cocktail of Alexa Fluor® 568-conjugated goat anti mouse IgG (Molecular Probes) and Alexa Fluor® 488-conjugated goat anti mouse IgG (Molecular Probes). After further washing, sections were stained with TO-PRO®-3, coverslipped with VECTASHIELD® (Vector) and observed with a laser-scanning confocal fluorescence microscope (LSM5 PASCAL, Carl Zeiss).

Biochemical analysis

Mouse brains were homogenized in 20 volumes (w/v) of Buffer A, then spun at 100 000*g* for 30 min at 4°C, and the supernatant was retained as buffer-soluble fraction. The pellet was homogenized in 20 volumes of Buffer A containing 1% Triton™ X-100 and incubated for 30 min at 37°C. After centrifugation at 100 000*g*, the Triton™-insoluble pellet was further homogenized in Buffer A containing 1% sarkosyl and incubated at 37°C for 30 min. Samples were spun at 100 000*g* for 30 min. The sarkosyl-pellet was sonicated in 30 mM Tris-HCl, pH 7.4, and used for immunoblotting as sarkosyl-insoluble fraction. The samples were subjected to SDS-PAGE on 12.5% polyacrylamide gel and proteins were electrotransferred onto a polyvinylidene difluoride membrane, probed with appropriate antibodies and detected as described (Nonaka *et al.*, 2009).

Behavioural tests

Open field test

Each mouse was placed in the centre of the open field apparatus (25-cm diameter). Activity was measured by SUPERMEX system (Muromachi Kikai) over 90-min period and analysed by CompACT AMS software ver.3 (Muromachi Kikai). Total activity was measured by counting the number of photobeam interruptions over every 5-min period.

Wire hang test

Neuromuscular strength was tested with a wire hang test. The mouse was placed on a wire mesh, waved gently so that the mouse gripped the wire and then inverted. Latency to fall was recorded with a 300-s cut-off time.

Rotarod test

The Rotarod test, using an accelerating Rotarod (Muromachi Kikai), was performed by placing mice on 9-cm diameter rods and measuring the time each animal was able to maintain its balance on the rod. We used 9-cm rods to make the test more sensitive to motor skill learning (Shiotsuki *et al.*, 2010). The speed of the rotarod accelerated from 0 to 40 rpm over a 5-min period.

Y-maze test

Y-maze apparatus (Muromachi Kikai) consisted of three arms (40 cm × 3 cm) made of grey plastic joined in the middle to form a Y shape. Mice were placed into one of the arms of the maze and allowed to explore freely the maze for an 8-min session. The alternation between arms was recorded.

Intranasal administration of abnormal α-synuclein fibrils

Twenty micrograms of recombinant α-synuclein monomer or preformed fibrils, or 10 μl of insoluble fraction of dementia with Lewy bodies brain, was administered intranasally once a week for 1 month to 10-week-old female C57BL/6J mice (soluble mouse α-synuclein, soluble human α-synuclein, mouse α-synuclein fibrils, human α-synuclein fibrils and dementia with Lewy bodies extracts, $n = 5$ per group). At 21 months after the last administration, mice were anaesthetized with pentobarbital sodium and killed by perfusion with phosphate buffer (pH 7.4) and 4% paraformaldehyde in 0.1% phosphate buffer. Brains were cryosectioned and immunostained as described above.

Results

To investigate whether insoluble α-synuclein fibrils can propagate *in vivo*, we injected recombinant human α-synuclein fibrils into the substantia nigra in the right cerebral hemisphere of C57BL/6J mice. α-Synuclein fibrils were prepared using highly purified recombinant α-synuclein (Supplementary Fig. 1A) by incubation with shaking. Formation of the fibrils was confirmed by electron microscopy (Supplementary Fig. 1B) and thioflavin S assay (data not shown). The fibrils were then collected by ultracentrifugation, sonicated and used for injection. Abnormal phosphorylated α-synuclein-positive structures were observed in the brains of mice injected with human α-synuclein fibrils at 15 months after inoculation (Fig. 1). Phosphorylated α-synuclein pathology was distributed throughout the brain including substantia nigra, amygdala, dentate gyrus, hippocampal CA1-3, molecular layer of hippocampus, fimbria, stria terminalis, hypothalamus, somatosensory area, visual cortex, cingulate cortex and corpus callosum (Fig. 1). Phosphorylated α-synuclein-positive structures were also positive for anti-ubiquitin and p62 antibodies (Fig. 2A). Co-localization was confirmed by confocal microscopy (Fig. 2B and C), indicating that these structures have the same immunoreactive properties as Lewy bodies (Kuusisto *et al.*, 2001). By contrast, no phosphorylated α-synuclein, ubiquitin or p62-positive pathology was observed in the brains of mice injected with soluble human α-synuclein (Supplementary Fig. 2). Remarkably, despite the unilateral injection of α-synuclein fibrils, phosphorylated α-synuclein-positive pathology appeared bilaterally (Fig. 3A). In the right hemisphere (injected side), phosphorylated α-synuclein pathology was seen abundantly in dentate gyrus and amygdala, whereas in the left hemisphere no pathology was seen in amygdala and only sparsely in dentate gyrus (Fig. 3A). These results strongly suggest that α-synuclein pathology propagates throughout the brain from the injection site. To understand the spreading pathway of phosphorylated α-synuclein pathology, we investigated in detail the distribution in four coronal sections at 15 months after inoculation (Fig. 3B). Near the injection level (bregma −3.40 mm), abundant phosphorylated α-synuclein pathology was present in substantia nigra, hippocampus, external capsule and entorhinal cortex in right hemisphere, whereas in the left hemisphere, sparser pathology was detected in hippocampus and external capsule (Fig. 3B). By contrast, at the level of 0.02 mm from bregma (3 mm anterior to the injection level), phosphorylated α-synuclein pathology was concentrated in stria terminalis, septal nucleus and cingulate, motor and somatosensory cortex in the right hemisphere. In the left hemisphere, phosphorylated α-synuclein pathology was detected only in septal nucleus (Fig. 3B). These results suggest that phosphorylated α-synuclein pathology does not spread by simple diffusion and the propensity to accumulate phosphorylated α-synuclein seems to differ among brain regions. The time course of spreading of phosphorylated α-synuclein pathology was analysed by immunohistochemistry and summarized in Table 1. Table 1 clearly indicates that induction of phosphorylated α-synuclein pathology in wild-type mice is time- and brain region-dependent. No signs of astrogliosis and inflammation were observed in human α-synuclein fibril-injected mice compared with soluble-human α-synuclein-injected mice at 15 months after injection (Supplementary Fig. 3).

To clarify which α-synuclein species accumulated in the mice, and when, we performed immunoblot analysis with LB509 and anti-mouse synuclein antibodies, which specifically recognize human α-synuclein and mouse α-synuclein, respectively. The antibody specificities are shown in Supplementary Fig. 1B. At a few

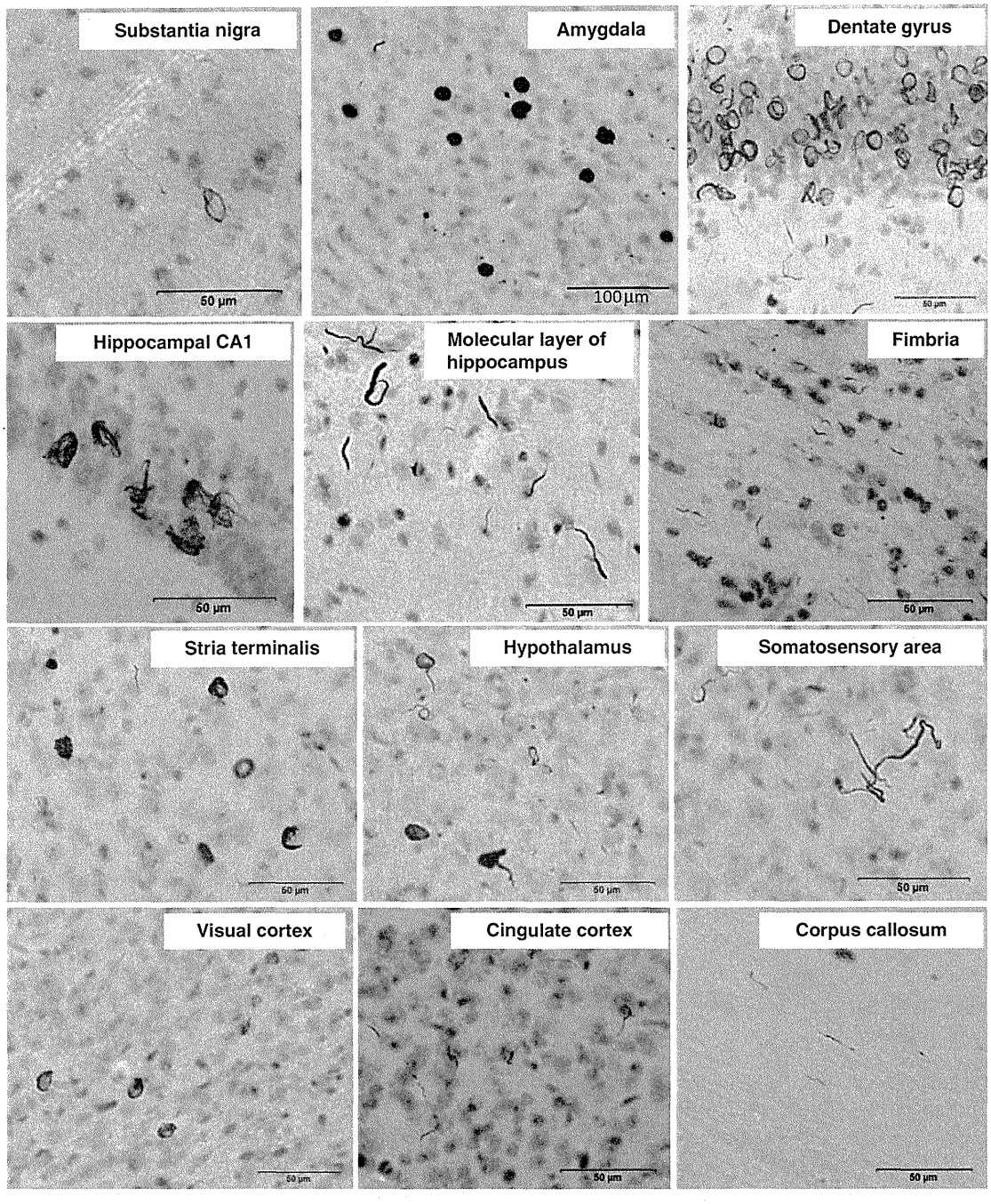

Figure 1 Induction of phosphorylated α-synuclein pathology in wild-type mouse brain injected with human α-synuclein fibrils, observed at 15 months after injection. Sections were immunostained with anti-phosphorylated α-synuclein antibody, 1175. The shapes of phosphorylated α-synuclein-positive structures differed among brain areas. Ring-like and Lewy neurite-like structures were observed in substantia nigra, hippocampus, hypothalamus, somatosensory area, visual cortex, cingulate cortex and corpus callosum, whereas Lewy body- and Lewy neurite-like structures were observed in amygdala and stria terminalis.

hours after injection (Day 0), injected recombinant human α-synuclein fibrils were detected in the sarkosyl-insoluble fraction of the right and left hemispheres by LB509 antibody, suggesting that injected human α-synuclein fibrils in the extracellular space spread quickly throughout the brain. However, at 7 days after injection, the human α-synuclein immunoreactivities had disappeared, and did not reappear at 30 or 90 days after injection (Fig. 4). At 90 days after injection, anti-phosphorylated α-synuclein-positive 15, 20, 30 and 35 kDa bands were detected in the sarkosyl-insoluble fractions. This band pattern is indistinguishable from that of pathological α-synuclein in dementia with Lewy bodies brain (Fig. 4). The 15, 20, 30 and 35 kDa bands correspond to α-synuclein monomer, mono-ubiquitinated α-synuclein, dimer and ubiquitinated dimer, respectively. Most

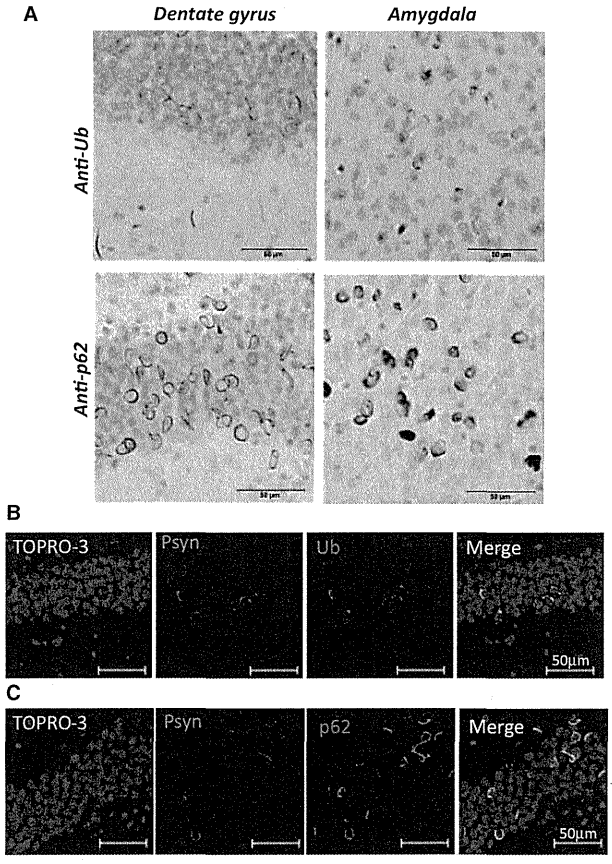

Figure 2 α-Synuclein pathology in fibril-injected mice brain was immunoreactive for ubiquitin (Ub) and p62. (**A**) Staining of dentate gyrus and amygdala of fibril-injected mice at 15 months after injection, using anti-ubiquitin (*upper*) and p62 (*lower*) antibodies. Abundant ubiquitin- and p62-positive pathology can be seen. (**B** and **C**) Double-labelled immunofluorescence of dentate gyrus for phosphorylated α-synuclein (Psyn) and ubiquitin (**B**) or p62 (**C**). Phosphorylated α-synuclein-positive structures were co-localized with ubiquitin and p62.

interestingly, anti-mouse α-synuclein strongly labelled the sarkosyl-insoluble phosphorylated α-synuclein-positive bands at Day 90, but these were not immunostained with LB509. These results clearly show that endogenous mouse α-synuclein is accumulated as phosphorylated and ubiquitinated forms. Immunohistochemical analysis with anti-tyrosine hydroxylase suggested that dopaminergic neurons are retained in substantia nigra of human α-synuclein fibril-injected mice at 6 months after injection (Fig. 5A and B). However, dramatic loss of the neurotransmitter enkephalin was observed in globus pallidus and amygdala central nucleus, where abundant phosphorylated α-synuclein-positive structures are detected (Fig. 5C and D). These data suggest that neuronal dysfunction occurs without apparent neuronal loss. We also performed behavioural analyses of mice injected with soluble human α-synuclein monomers or human α-synuclein fibrils. However, significant differences were not observed in open field test, wire hang test, rotarod test and Y-maze test (Supplementary Fig. 4) at 6 months after injection.

Next, we tested whether fibrils composed of recombinant mouse α-synuclein can induce α-synuclein pathology more efficiently than those composed of human α-synuclein, because the sequences of human and mouse α-synuclein are slightly different (Supplementary Fig. 5), and there could be a species difference. Mouse α-synuclein complementary DNA was cloned, and the protein was expressed in *Escherichia coli* and purified. Fibrils or soluble mouse α-synuclein were inoculated into substantia nigra of wild-type mouse brains and the pathology was evaluated. Strikingly, all the mice injected with mouse α-synuclein fibrils developed phosphorylated α-synuclein pathology in the injected side of the brain, whereas no pathology was detected in mice injected with soluble mouse α-synuclein (Table 2). The phosphorylated α-synuclein pathologies were basically the same as those of mice injected with human α-synuclein fibrils (data not shown). The efficiency of the induction of phosphorylated α-synuclein pathology by human α-synuclein fibrils was ~90% (Table 2), which is quite high, but slightly lower than that with mouse α-synuclein fibrils, suggesting that there may be a small species difference between mouse and human α-synuclein.

Finally, we tested whether pathological α-synuclein deposited in the brains of patients has similar prion-like properties in brains of wild-type mice. Surprisingly, pathological α-synuclein-enriched fractions also induced phosphorylated α-synuclein-positive pathologies in various areas of brain, including the substantia nigra, amygdala, hippocampus, striatum, hypothalamus, somatosensory area, motor cortex, piriform cortex and superior colliculus (Fig. 6). In brains of these mice, the phosphorylated α-synuclein-positive pathologies mostly resembled Lewy neurite-like structures. Lewy body-like pathology was detected only in amygdala and piriform cortex. The percentage of mice that developed phosphorylated α-synuclein pathology in the injected side of the brains was 50% in the group injected with insoluble phosphorylated α-synuclein of dementia with Lewy bodies brains, which is less than that in mice injected with recombinant α-synuclein fibrils (Table 2). Thus, these results demonstrate that inoculation of either pure synthetic recombinant α-synuclein fibrils or dementia with Lewy bodies brain extracts into wild-type mice can induce Lewy body/neurite-like phosphorylated α-synuclein pathology efficiently and reproducibly. Our results raise an important question, i.e. whether or not α-synuclein fibrils are transmissible among individuals. To test this possibility, we intranasally administered at high concentrarion of abnormal α-synuclein fibrils (preformed recombinant human or mouse α-synuclein fibrils) or the insoluble fraction from dementia with Lewy bodies brain to normal mice. However, no pS129-positive abnormal structures were detected in the brain at 21 months after the final administration (Supplementary Fig. 6), even with highly sensitive immunohistochemical staining, suggesting that the abnormal α-synuclein cannot pass through the nasal mucosa.

Discussion

In this study, we have shown that the inoculation of α-synuclein fibrils made of recombinant α-synuclein or dementia with Lewy bodies brain extracts into wild-type mouse brain is sufficient to

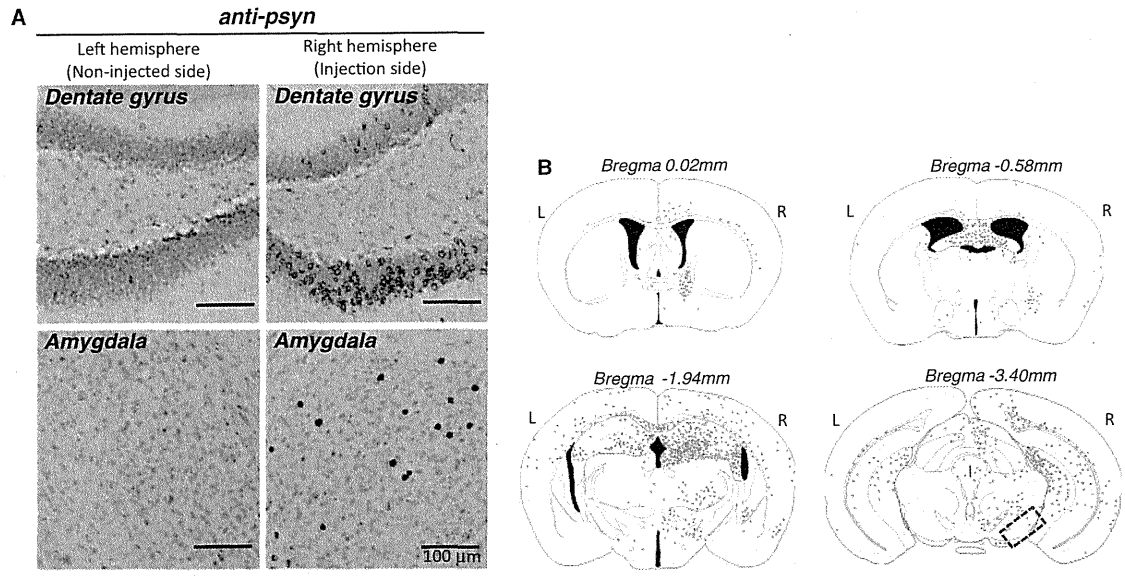

Figure 3 (**A**) Spreading of phosphorylated α-synuclein pathology on the contralateral side of mouse brain injected with α-synuclein fibrils. Staining of dentate gyrus and amygdala in the right hemisphere (injection side) and in the left hemisphere (non-injected side) with anti-phosphorylated α-synuclein (psyn) antibody, 1175, at 15 months after injection. (**B**) Distribution of phosphorylated α-synuclein pathology in human α-synuclein fibril-injected mouse brain at 15 months after injection (n = 24). Four coronal sections were stained with phosphorylated α-synuclein antibody, 1175. Red dots indicates Lewy bodies- and Lewy neurites-like pathology. Near the injection level (bregma -3.40 mm), abundant phosphorylated α-synuclein pathology was present in substantia nigra, hippocampus, external capsule, and entorhinal cortex in right hemisphere, whereas in the left hemisphere, sparser pathology was detected in hippocampus and external capsule. At the level of -1.94 mm from bregma, severe phosphorylated α-synuclein pathology was present in hippocampus, amygdala, corpus callosum, hypothalamus and motor, visual, somatosensory, auditory and piriform cortex in the right hemisphere, whereas moderate phosphorylated α-synuclein pathology was observed in corpus callosum, hippocampus, external capsule and motor, somasosensory and auditory cortex in the left hemisphere. At the level of -0.58 mm from bregma, phosphorylated α-synuclein pathology was detected in amygdala, corpus callosum, fimbria, fornix, hypothalamus, striatum and somatosensory and piriform cortex in the right hemisphere, whereas in the left hemisphere, the pathology was present in corpus callosum, fimbria, fornix, hypothalamus and striatum. At the level of 0.02 mm from bregma, phosphorylated α-synuclein pathology was concentrated in stria terminalis, septal nucleus and cingulate, motor and somatosensory cortex in the right hemisphere. In the left hemisphere, phosphorylated α-synuclein pathology was detected only in septal nucleus. Dashed box indicates substantia nigra (injection site). L = left hemisphere of brain; R = right hemisphere.

cause the appearance of Lewy body/neurite-like α-synuclein pathology *in vivo*. Similar work was recently published by Luk *et al.* (2012*a*) but there are important differences between our study and theirs. Luk *et al.* (2012*a*) showed that only inoculation of synthetic mouse α-synuclein fibrils into wild-type mouse brain induced synuclein pathology. In our present study, we inoculated not only fibrils made of recombinant mouse α-synuclein but also ones from human α-synuclein fibrils, and importantly also insoluble α-synuclein from dementia with Lewy bodies brains, into wild-type mouse brain. This is the first report showing efficient induction of α-synuclein pathology by inoculation of material from human brain. Furthermore, our biochemical analyses clearly demonstrate that endogenous mouse α-synuclein is converted into abnormal form and deposited in neurons of the brain through a prion-like mechanism or by seed-dependent aggregation by crossing the species barrier (Fig. 4). Since soluble α-synuclein never induced such pathology (Supplementary Fig. 2), we can conclude that the structural difference between soluble and filamentous forms of α-synuclein, i.e. cross-β structure in the α-synuclein fibrils (Serpell *et al.*, 2000) is critical for the pathogenesis. It has been reported that recombinant α-synuclein fibrils enhance the initiation and progression of α-synuclein pathology in transgenic mice overexpressing mutant α-synuclein (Mougenot *et al.*, 2012; Luk *et al.*, 2012*b*) and wild-type mice (Luk *et al.*, 2012*a*). In those models, α-synuclein pathology appeared at 90 days after inoculation. In our mouse model, abnormal phosphorylated α-synuclein pathology was also detected at 90 days after injection (Fig. 4 and Table 1), suggesting that it takes about this length of time for the formation of abnormal phosphorylated α-synuclein pathology *in vivo* after the seeding procedure. Despite a diffusion of injected exogenous α-synuclein fibrils to the bilateral sides of brain within a few hours after injection (Fig. 4), phosphorylated α-synuclein pathology seems to be initiated in the injected side and to spread from the injected side to the non-injected side in a time-dependent manner (Table 1). Thus, it is reasonable to speculate that exogenous fibrils enter neurons at the injection site as a result of infusion pressure, a temporary high concentration, or some other mechanism, and then the pathological process starts to develop from these cells.

Propagation patterns of pathology in the inoculated mice were basically identical regardless of the species of injected seeds (i.e. recombinant human α-synuclein fibrils, mouse α-synuclein fibrils or

Table 1 Semi-quantitative grading of α-synuclein pathology in mice injected with human α-synuclein fibrils

			Non-injection side (left hemisphere)			Injection side (right hemisphere)		
			Time from injection (days)			Time from injection (days)		
			90	*180*	*450*	*90*	*180*	*450*
Bregma	0.02 mm	Stria terminalis	–	–	–	–	+ +	+ + +
		Striatum	–	+	+	+	+ +	+ +
		Cigular cortex	–	–	–	–	+	+
		Septal nucleus	–	–	–	–	+	+
Bregma	−0.58 mm	Corpus callosum	–	–	+	–	–	+ +
		Fornix	–	+	+ +	–	+	+ +
		Hippocampal commissure	–	+	+ +	–	+	+ +
		Amygdala	–	–	–	+	+ + +	+ + +
		Globus pallidus	–	+	+	–	+	+ +
		Striatum	–	–	+	+	+	+
		Somatosensory area	–	–	+	–	+	+
		Insular cortex	–	–	–	+	+	+
Bregma	−1.94 mm	Corpus callosum	–	–	+ +	–	–	+ +
		Hippocampus	–	+	+ + +	+	+ +	+ + +
		Habenular nucleus	–	–	+	–	–	+ + +
		Fimbria	–	+	+ + +	–	+	+ + +
		Amygdala	–	–	–	+ +	+ + +	+ + +
		Hypothalamus	–	–	+	+	+	+ +
		Thalamus	–	–	–	–	–	+
		Visual cortex	–	–	+	–	+	+ +
		Somatosensory area	–	+	+	–	+	+ +
		Auditory cortex	–	–	+	+	+	+ +
		Piriform cortex	–	–	+	+	+	+ +
		External cupsule	–	–	+	–	–	+ +
Bregma	−3.40 mm	Substantia nigra	–	–	–	+	+	+
		Hippocampus	–	+	+ +	+	+ +	+ +
		Superior colliculus	–	+	+	–	+	+ +
		External cupsule	–	–	+	–	–	+
		Visual cortex	–	–	–	+	+	+
		Auditory cortex	+	+	+	+	+ +	+ +
		Entorhinal cortex	–	+	+	+	+ +	+ +

Four coronal sections were stained with anti-phosphorylated α-synuclein antibody at 90, 180 or 450 days after injection. Grading of α-synuclein pathology was performed as follows: –, none; +, slight; + +, moderate; + + +, severe. At 90 days after injection, small amounts of phosphorylated α-synuclein-positive structures were observed in substantia nigra, amygdala, striatum, hypothalamus, hippocampus, and stria terminalis in the right hemisphere of brain (injected side), but very few Lewy neurites were detected in cortex in the left hemisphere. At 180 days post-injection, the amount of phosphorylated α-synuclein-positive pathology was increased and was more widely spread in the right hemisphere, while in the left hemisphere, little phosphorylated α-synuclein pathology was apparent in hypothalamus, hippocampus, striatum or globus pallidus. At 450 days (15 months) after injection, phosphorylated α-synuclein pathology had spread throughout the right hemisphere and the left hemisphere.

dementia with Lewy bodies brain extracts), but extracts of brains with dementia with Lewy bodies showed lower propagation efficiency than recombinant fibrils (Table 2). This relatively low efficiency may be explained by the lesser amount of abnormal α-synuclein contained in the dementia with Lewy bodies brain extracts. Comparison of human α-synuclein fibrils and mouse α-synuclein fibrils indicated that mouse α-synuclein fibrils showed slightly higher efficiency (Table 2). *In vitro* experiments also indicated that mouse α-synuclein fibrils promote fibrillization of the soluble mouse α-synuclein monomer faster than human α-synuclein fibrils (Supplementary Fig. 7). It is well known that prion propagation can cross the species barrier (Prusiner, 1993) and the efficiency of propagation depends on the amino acid sequences of prion proteins. In the case of α-synuclein, mouse α-synuclein and human α-synuclein share 95% amino acid sequence homology (Supplementary Fig. 5), and this may be the reason why endogenous mouse α-synuclein is capable of aggregation by inoculation of human α-synuclein fibrils. Another factor may be that mouse α-synuclein protein has a threonine residue at amino acid position 53 (Supplementary Fig. 5), which is known as an aggregation-prone mutation in familial Parkinson's disease (Polymeropoulos *et al.*, 1997).

Time course analyses of the pathology in these mice (Table 1) showed that at 90 days after injection, phosphorylated α-synuclein pathology was mainly observed near the injection level, but also seen in striatum, amygdala, stria terminalis and dentate gyrus: areas far from the injection site had developed pathology. The striatum and the amygdala central nucleus have projections from substantia nigra, and the stria terminalis serves as a major output pathway of the amygdala (Supplementary Fig. 8). Although the

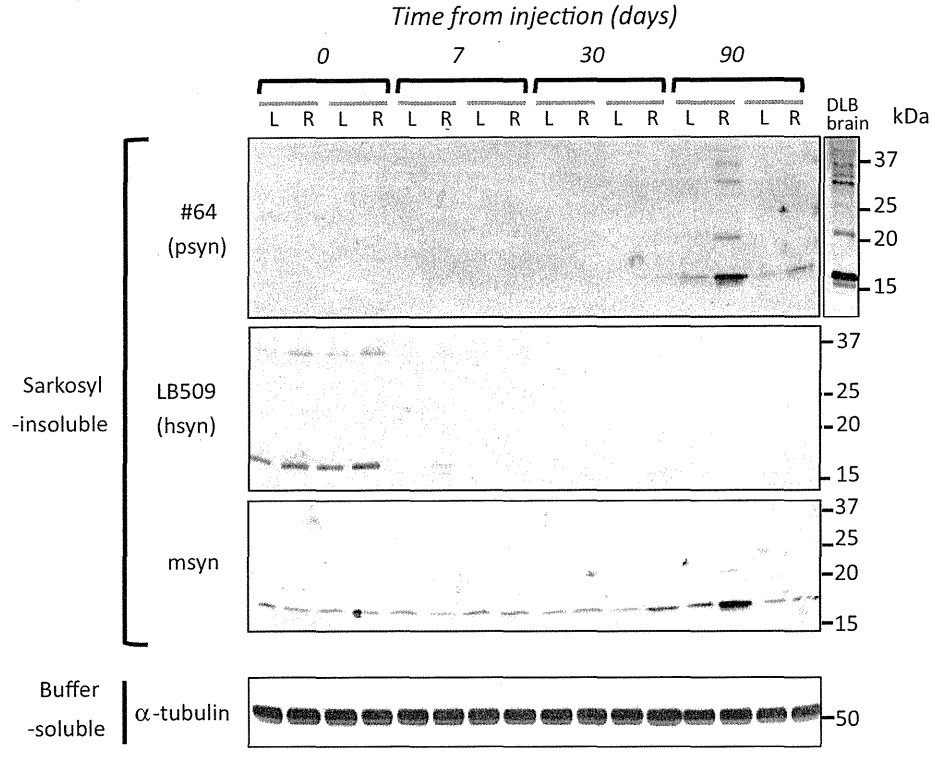

Figure 4 Endogenous mouse α-synuclein was aggregated in wild-type mouse brain injected with human α-synuclein (hsyn) fibrils. The brain was divided into two parts at the longitudinal fissure of the cerebrum. Sarkosyl-insoluble fractions were obtained from the right and left hemispheres, and analysed by immunoblotting with #64, LB509 or anti-mouse α-synuclein (msyn) antibodies. Representative images are shown (n = 14). Sarkosyl-insoluble phosphorylated α-synuclein (psyn) started to accumulate, predominantly in the right hemisphere, at 90 days after injection. It was composed of endogenous mouse α-synuclein, not exogenous human α-synuclein.

dentate gyrus does not have direct projection to substantia nigra, regions connecting with dentate gyrus (i.e., hippocampal CA1, CA3, entorhinal cortex, fimbria, fornix and hippocampal commissure) also showed moderate pathology (Table 1). These results may indicate that α-synuclein pathology propagates unidirectionally through the neural circuit (Supplementary Fig. 8). Spread of pathology from the right hemisphere to the left hemisphere might occur via the corpus callosum, hippocampal commissure, etc., connecting with the contralateral side of the brain (Fig. 3B and Table 1). Phosphorylated α-synuclein pathology in our mouse model was mainly observed in neurons and was hardly detected in glial cells, while the band pattern of sarkosyl-insoluble phosphorylated α-synuclein in mice was indistinguishable from that of dementia with Lewy bodies brains (Fig. 4), where phosphorylated α-synuclein pathology was mainly seen in neurons. Although the mechanism remains to be clarified, exogenous α-synuclein fibrils may enter cells through a selective mechanism(s), such as neuron-specific receptors. Alternatively, differences in expression levels of endogenous α-synuclein or cellular environments may also be important for formation of the pathology, even if abnormal α-synuclein has already entered the cells.

Luk *et al.* (2012a) reported dopaminergic neuronal loss and motor dysfunction (by Rotarod test and wire hang test) in wild-type mice injected with mouse α-synuclein fibrils at 6 months after inoculation into striatum. In contrast, our human α-synuclein or mouse α-synuclein fibril-injected mice did not

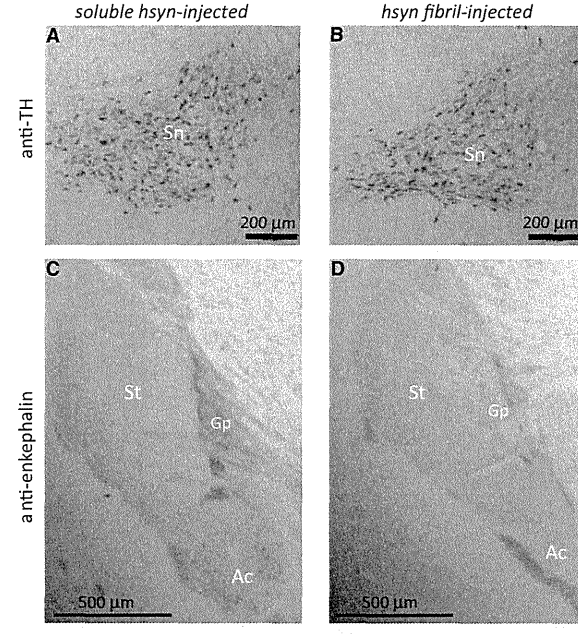

Figure 5 Fibril-injected mice show apparent reduction of a neurotransmitter enkephalin in amygdala central nucleus and globus pallidus at 15 months after injection. Brain sections were stained with anti-tyrosine hydroxylase (TH) (**A** and **B**) and anti-enkephalin (**C** and **D**) antibodies. Ac = amygdala central nucleus; Gp = globus pallidus; Sn = substantia nigra; St = striatum.

Table 2 Comparison of propagation efficiency in mice at 15 months after injection

Injection samples		Right hemisphere (injection side) anti-psyn	anti-ubiquitin	anti-p62	Left hemisphere (non-injected side) anti-psyn
Soluble human α-syn	(*n* = 8)	0/8 (0%)	0/8 (0%)	0/8 (0%)	0/8 (0%)
Insoluble human α-syn fibril	(*n* = 24)	22/24 (91.6%)	21/24 (87.5%)	22/24 (91.6%)	19/24 (79.2%)
Soluble mouse α-syn	(*n* = 4)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
Insoluble mouse α-syn fibril	(*n* = 8)	8/8 (100%)	7/8 (87.5%)	8/8 (100%)	8/8 (100%)
DLB brain extracts	(*n* = 14)	7/14 (50%)	0/14 (0%)	5/14 (35.7%)	1/14 (7.1%)

In the right hemisphere, mice showing immunopositive structures for anti-phosphorylated α-synuclein (psyn), ubiquitin (Ub) or p62 were counted. In the left hemisphere, mice showing immunopositive structures for anti-phosphorylated α-synuclein were counted. Values show number of immunopositive mice/total mice, with percentage of immunopositive mice. DLB = dementia with Lewy bodies.

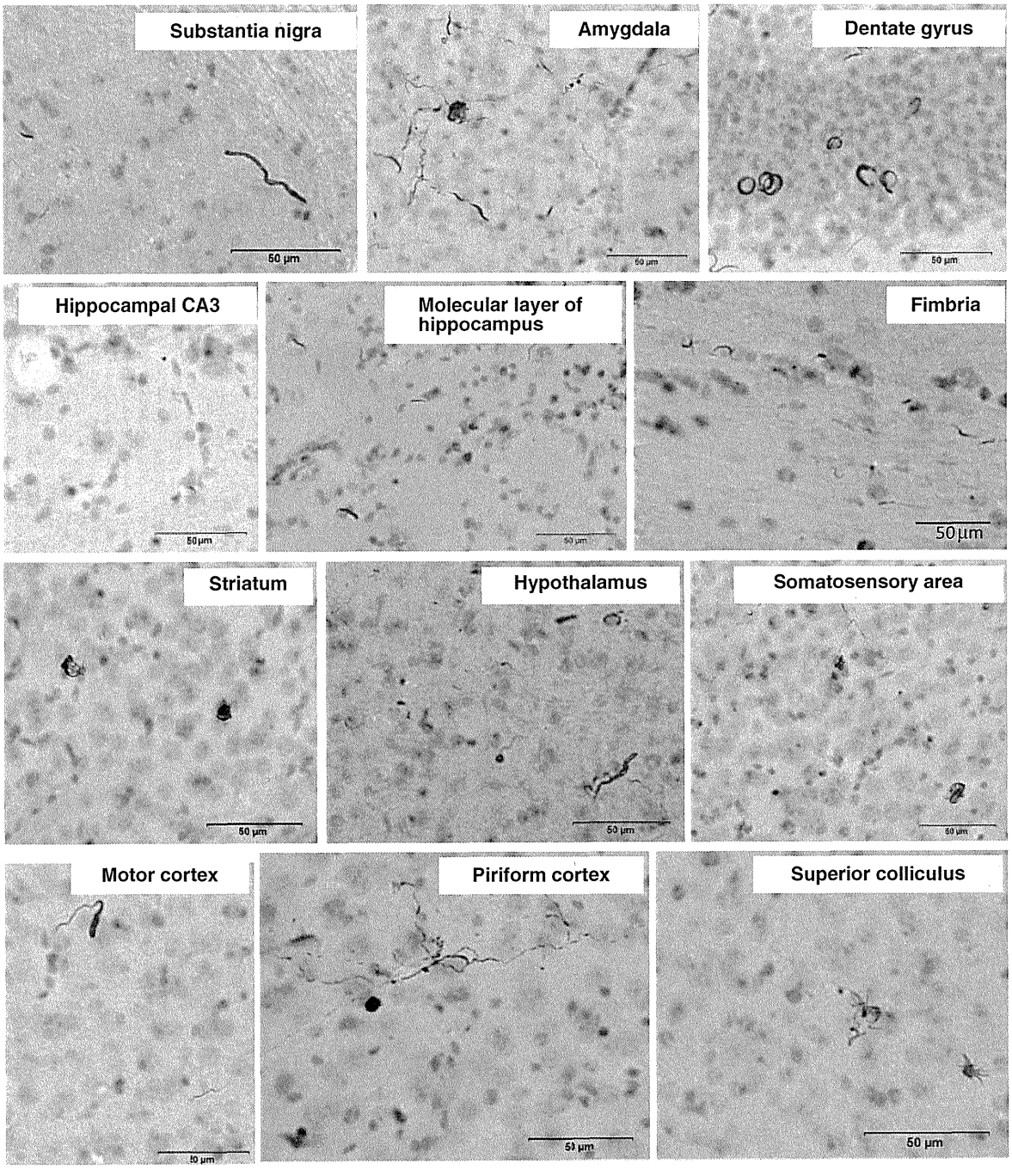

Figure 6 α-Synuclein pathology in wild-type mice brain injected with dementia with Lewy bodies-insoluble fraction observed at 15 months after injection. Sections were immunostained with anti-phosphorylated α-synuclein antibody, 1175.