平成23年度研究成果の刊行に関する一覧表

書籍

書籍　著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
深谷　親、山本隆充、片山容一	パーキンソン病に対する視床下核DBS	寺本　明、新井　一、塩川芳昭、大畑建治編	Neurosurgery NOW 機能的脳神経外科手術の基本	メジカルビュー社	東京	2011	pp12-23

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Miyagi Y, Morooka K, Fukuda T, Okamoto T	Three-dimensional reconstruction of human brain histological section and the development of digital brain atlas of the Japanese.	Proceedings of the 3rd International Symposium on Digital Manufacturing		153-158	2011
Taira T.	[Update on multimodal neurosurgical management of dystonias].	脳と発達	43-3	183-8	2011
Bronte-Stewart H, Taira T, Valldeoriola F, Merello M, Marks WJ Jr, Albanese A, Bressman S, Moro E.	Inclusion and exclusion criteria for DBS in dystonia.	Movement Disorders	26(S1)	S5-16	2011
Thobois S, Taira T, Comella C, Moro E, Bressman S, Albanese A.	Pre-operative evaluations for DBS in dystonia.	Movement Disorders	26(S1)	S17-22	2011
Yamada K, Sakurama T, Soyama N, Kuratsu J	GPi-pallidal stimulation for Lance-Adams syndrome	Neurology	76	1270-1272	2011

山田和慶、長谷川雄、倉津純一	不随意運動症に対する定位脳手術とその治療ターゲット②	脳神経外科速報	21	56-63	2011
山本隆充、深谷親、片山容一	機能神経外科の現在と未来	神経内科	76	563-571	2011
深谷　親、山本隆充、片山容一	脳深部刺激療法（Deep brain stimulation）	Clinical Neuroscience	29	415-418	2011
深谷　親，下田健太郎，渡辺　充，森下登史，角光一郎，大高稔晴，大渕俊樹，加納利和，小林一太，大島秀規，山本隆充，片山容一	ジストニアに対する脳深部刺激療法の長期成績	機能的脳神経外科	50(1)	28-29	2011

Ⅳ. 研究成果の刊行物・別刷

デジタル画像処理技術を用いた脳座標アトラス作成法

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砂川　賢二[1]／飛松　省三[4]／吉浦　敬[5]／佐々木　富男[2]

Application of digital imaging technique for the construction of stereotactic human brain atlas

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Abstract: An ideal human brain atlas must provide the universal coordinates of standard healthy brain. Morpholoy of human brain contains considerable inter-individual variety; however, the classical human brain atlases have been made from a limited number of materials. A large organ such as human brain is subject to mechanical deformation and the production of stereotactic human brain atlases is still time-consuming and needs special instruments, environments and skills. To achieve both the spatial consistency and high histological quality for stereotactic human brain atlases, we have established a novel technique for constructing the stereotactic brain atlas using a formalin-fixed cadaver brain of the Japanese, blade-oscillation microslicing and digital imaging techniques. Our method enabled the accurate reconstruction of human brain histological slices with the three-dimensional consistency necessary for the stereotactic atlases of human brain, as well as the successful preservation of original macroscopic shape and cytoarchitecture.

Keywords: Human brain atlas; Histological section; Stereotactic functional neurosurgery

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機能的脳神経外科 49(2010)82-83

はじめに

個人差の大きい脳を一つのアトラスで表し手術で用いるのは明らかに危険である。なるべく多数の脳標本から形状を平均化した標準脳によるアトラスや，各個体形状に応じて内部座標を補正できるテイラーメイド脳アトラスが望まれる。しかしヒト脳のように柔らかく大きな臓器を，変形させることなく切片化し，なおかつ良質の組織像を得るためには，特殊な機材と熟練，そして膨大な時間を要する。そのため，視床や基底核・脳幹部など深部構造に限られた狭い範囲を座標化するか，機械的変形を無視して脳半球全体を座標化するかの選択に迫られる。我々は三次元的整合性と細胞構築を両立させる，より簡便なヒト脳アトラスの作成法を確立した。

方　法

解剖実習に用いられる献体から選別された脳(89歳，男性)に関しては，本研究への利用について御遺族の承諾を得た。これらの献体は死後10%ホルマリン5リットルを大腿動脈から注入し12時間経過した後，エタノール・ホルマリン溶液に数週間浸潤させた後，実習開始まで4℃に保存したものである。頭蓋から摘出した脳はホルマリンでよく固定されており，萎縮は軽度で肉眼的に健康な脳であった。くも膜や脳表血管を除去した後，非接触型3次元デジタイザーを用いて脳全体の初期形状を記録した。正中から脳半球に分割し，全体を3% agarに包埋し，正確に平行切断し1 cm厚にブロック化した。一度にマイクロスライスできるブロックサイズは最大7×7 cmであるため，必要に応じてさらに分割した。次にagarを除去し，ブロックを10%ゼラチン溶液に入れ気泡を除去して包埋後，ブロック全体を4%パラフォルムアルデヒドで一晩固定し，ゼラチンの外枠部分4ヵ所に垂直に針を刺入しマーキングした。振動刃マイクロスライサーを用いて100 μmの切片を作成した。組織切片をニッスル染色および髄鞘染色して，スキャナで電子化し，3Dニューロン再構築解析ソフトウェア (NeuroLucida TM) を用い用手的に組織構造の輪郭をトレースし，3D再構築した。再構築画像を初期形状と比較して標本作製に伴う変形の有無を検証し，さらに細胞構築の保存状況を検証した。

結　果

Fig.1に脳ブロックの一つから得た再構築像を異なる方向から示す。レンダリング等の改変は加えていない。ヒト大脳皮質に特有の深い脳溝や複雑な脳回が正確に再現

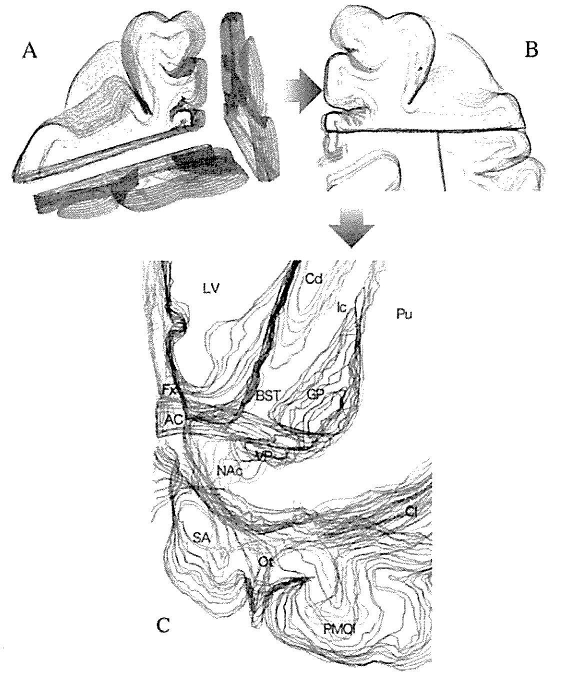

Fig.1 The contours of serial histological microslices were successfully reconstructed (A). The blocks could be put side by side and assembled into large block without any correction or revision (B). Contours of anterior basal ganglia could be clearly demonstrated according to cytoarchitecture (C). (LV, lateral ventrcle; Cd, caudate nucleus; Ic, internal capsule; Pu, putamen; Fx fornix; Ac, anterior commissure; BST, bed nucleus of stria terminalis; GP, globus pallidus; VP, ventral pallidum; NAc, nucleus accumbens; SA, subcallosal area; Ot, olfactory tract; Cl, claustrum; PMOl, posteromedial orbital lobule)

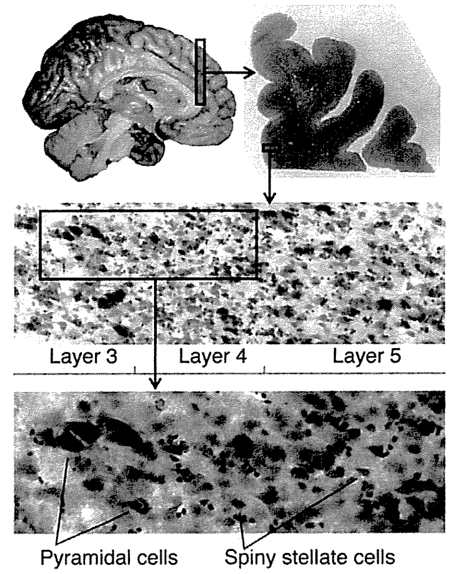

Fig.2 Nissl-staining of the section used for brain atlas reconstruction. Histological sections included in this partially reconstructed brain atlas provided the excellent preservation of area-specific cytoarchitecture of cerebral cortex; Brodmann area 32 was discriminated by the presence of large pyramidal neurons in deep layer III and spiny stellate neuron in layer IV in cadaver brain.

されている(Fig.1-A)。3つのブロックから得たトレース像をそれぞれ拡大縮小や変形することなく限りなく近づけ，一つの像に組み合わせると，ブロック断面で矛盾はなく高い整合性が得られた(Fig.1-B)。このブロック内部の様々な領域はトレースの過程で組織の顕微鏡観察により同定できた(Fig.1-C)。

さらに再構築に用いた献体脳の帯状回 Brodmann area 32 の光顕像(ニッスル染色，Fig.2)では，第Ⅲ層の大錐体細胞や第Ⅳ層の有棘星状細胞が明瞭に確認され，大脳皮質層構造に特異的な細胞構築がよく保たれていることがわかった。

考　察

ヒト大脳アトラスは，Schaltenbrand & Wahren アトラス[1]が最も広く手術支援ソフトウェア(SurgiPlan, FrameLink, StereoPlan, Cerefy など)に利用されている。しかし実際の脳形状は個人差に富み，Talairach グリッドで補正できるほど容易ではないため，機能的定位脳手術においては侵襲的な微小電極記録で標的神経核を生理学的に確認する作業が求められる。個体差に対応できる正確なアトラスであれば，微小電極記録という侵襲的操作を行わずに機能的定位脳手術が可能となるはずである。Yelnik ら[2]は，スライス前に MRI で脳形状を記録し，作成した凍結固定スライス標本を検証し，補正に用いた。作製されたスライス標本では脳室が潰れ肉眼的に大きく変形し，特に周辺部(脳表や脳室壁)は凍結固定に伴う損傷が激しい。この方法でも基底核や視床に限局したアトラスは作製可能かもしれないが，細胞構築が保たれていないため亜核などの同定は不可能である。このような先行研究からも，ヒト大脳という巨大で柔らかい組織を変形なく細胞構築を温存し全体を連続標本にすることの困難さがわかる。

我々の開発した方法では，脳は頭蓋から取り出す前に(in situ)十分ホルマリン固定されている上，3次元デジタイザーで正確に脳表の形状保存ができ，agar で周囲を支持することでブロック化に伴う肉眼的変形を防ぎ，振動刃マイクロスライサーで細胞構築を保ちつつスライスすることにより，3次元的整合性と美しい組織染色画像を両立できることがわかった。

ヒト脳アトラス作成の第一段階として，完全で普遍的な形状モデルを得ることが目標である。将来のニューロモデュレーションにおいて，様々な機能的脳疾患に対し，様々な治療標的(神経核や神経線維)が開発される可能性を想定すると，皮質や脳幹も含めた，脳全体の座標アトラスを作成することが望まれる。

結　論

デジタル画像処理技術を応用し，ブロック間やブロック・半球間の3次元的整合性が保たれ，標本作製に伴う変形が全くなく，細胞構築が非常に良く保たれた再構築画像を得ることに成功した。

文献

1) Schaltenbrand G et al: Atlas for stereotaxy of the human brain. Thieme, Stuttgart, 1977.

2) Yelnik J et al: A three-dimensional, histological and deformable atlas of the human basal ganglia I. Atlas construction based on immunohistochemical and MRI data. NeuroImage 34: 618-638, 2007.

ヒト脳座標アトラス作成におけるデジタル画像技術の応用

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抄　録：機能的定位脳手術ではヒト脳座標アトラス（Schaltenbrand & Wahren 1977）を参考に標的神経核の仮座標が定められることが多い。しかし個人差の大きい脳を一つのアトラスで表しその座標を機能的定位脳手術で用いるのは危険である。多数の脳標本から形状を平均化した標準脳でアトラスを作成し，これを各個体の脳形状に応じてnon-rigidに内部座標を補正できるテイラーメイド脳アトラスが望まれる。ヒト脳のように大きく柔らかな臓器を，変形させずに切片化し，なおかつ良質の組織像を得るためには，特殊な機材と熟練，そして膨大な時間を要する。そのため，視床や基底核・脳幹部など深部構造に限られた狭い範囲を座標化するか，機械的変形を無視して脳半球全体を座標化するかの選択に迫られる。我々は三次元的整合性と細胞構築を両立させる，より簡便なヒト脳座標アトラスの作成法を検討した。日本人の献体脳を用い，非接触型3次元デジタイザーで脳全体の初期形状を記録した後，正確な平行切断で1 cm厚にブロック化，振動刃ミクロトームを用いて100 μmの切片を作成，組織切片をニッスル染色後，スキャナで電子化し，3D再構築解析ソフトウェアで用手的に組織構造の輪郭をトレースし再構築した。これらの手法により標本作製に伴う機械的変形が全くない，三次元的整合性と細胞構築を両立させる，より簡便なヒト脳アトラスの作成法を確立できた。

索引用語：デジタル画像処理技術；ヒト脳座標アトラス；機能的定位脳手術；基底核

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はじめに

機能的定位脳手術は解剖学的標的設定と電気生理学的同定が必要とされる。古くから機能的定位脳手術の解剖学的指標として最も使用されてきた脳アトラスはSchaltenbrand & Bailey, Schaltenbrand & Wahren[13]やTalairach & Tournoux[14]で，これらをもとに手術用にデジタル化もなされている[11]。Schaltenbrand & Wahrenアトラス[13]では3方向からの肉眼組織画像が提示されているが，各切片が等間隔ではなく，観察方向によって個体が異なるため座標の整合性がない。また再構成した神経核は三次元的に不自然な形状を呈し，冠状断切片は交連間線に垂直ではなく7°傾斜している[6,7]。しかし最も実用的でない点は，個人の脳に適応するための変形が単純な変倍操作のみで自由度がないことである。その結果，臨床応用するには脳画像に合わせてかなりの縮尺調整を行う必要があるが，現在の変倍操作では個体差に対応した変形ができずテイラーメイド手術ができない。また同一個体内であっても殆どの場合左右対称ではなく，術前MRIで左右の脳半球～視

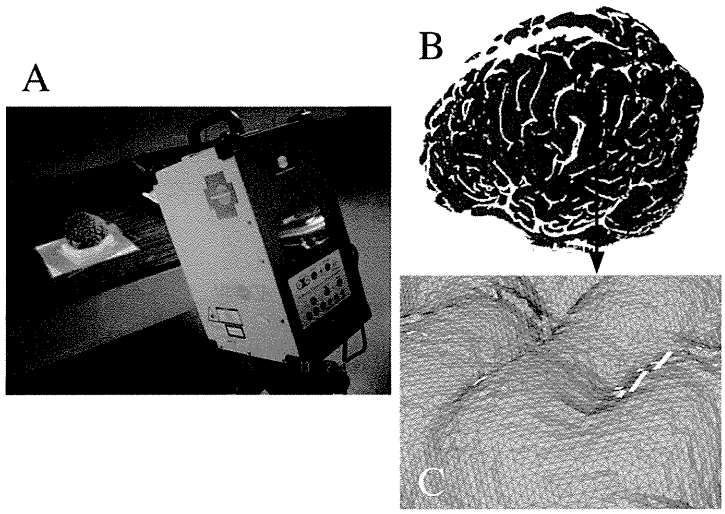

Fig.1 Digital recording of the surface data of human brain. (A) After removing the arachnoid membrane, the whole surface of the brain was scanned by a non-contact 3D digitizer (VIVID910, Konica, Minolta, Sakai, Japan). (B) The multiple range images acquired from different views were registered to convert them into a unified coordinate system. The Iterative Closest Point (ICP) algorithm was used to find the relationship between two different coordinates. (C) The mesh surface of the integrated brain model was reproduced with considerably high resolution and precision.

床・中脳にねじれ(回転変形)した症例に遭遇することも非常に多い。個人差の大きい脳形状を一つのアトラスで表し手術で用いるのは明らかに危険であり，なるべく多数の脳標本から形状を平均化した標準脳によるアトラスや，各個体の脳形状に応じて内部座標を補正できる，全く新しいテイラーメイド脳アトラスが望まれる[9]。しかしヒト脳のように柔らかく大きな臓器を，変形させることなく切片化し，なおかつ高画質の組織像を得るためには，特殊な機材と熟練，そして膨大な時間を要する。そのため視床や基底核・脳幹部など深部構造に限られた狭い範囲を精密に座標化するか，ある程度の機械的変形や細胞構築の描出を無視して脳半球全体を座標化するかの選択に迫られる。我々は三次元的整合性と細胞構築を両立させる，より簡便なヒト脳アトラスの作成法を確立した。

方　法

解剖実習に用いられる献体から選別された脳（89歳，男性）を用いた。献体の利用に関しては，御遺族に本研究の主旨を説明した上で，献体の脳半球を実習と同時に研究利用することに対して文書で承諾を得た。これらの実習用献体は死後10%ホルマリン5リットルを大腿動脈から注入し12時間経過した後，エタノール・ホルマリン溶液に数週間浸潤させた後，実習開始まで4℃に保存したものである。頭蓋から摘出した脳はホルマリンでよく固定されており，萎縮は軽度で肉眼的には健康な外観であった。くも膜や脳表血管を除去した後，非接触型三次元デジタイザ（VIVID 910, コニカミノルタ センシング, Fig.1-A）を用いて脳全体の初期形状を記録した。正中から脳半球に分割し，全体を3% agarに包埋し，正確に平行切断し1 cm厚にブロック化した。一度にスライスできるブロックサイズは最大7×7 cmであるため，必要に応じてさらに分割した。次にagarを除去し，ブロックを10%ゼラチン溶液に入れ気泡を除去して包埋後，ブロック全体を4%パラフォルムアルデヒドで一晩固定し，ゼラチンの外枠部分4ヵ所に垂直に針を刺入しマーキングした。振動刃マイクロスライサ（DTK-3000W, 堂阪）を用いて100 μmの切片を作成した。組織切片をニッスル染色および髄鞘染色して，スキャナで電子化し，3Dニューロン再構築解析ソフトウェア（NeuroLucida^{TM}, MicroBrightField Bioscience, Inc., USA）を用いて組織構造の輪郭を用手的にトレースし再構築した。再構築画像を初期形状と比較して標本作製に伴う変形の有無を検証し，さらに細胞構築の保存状況を検証した。

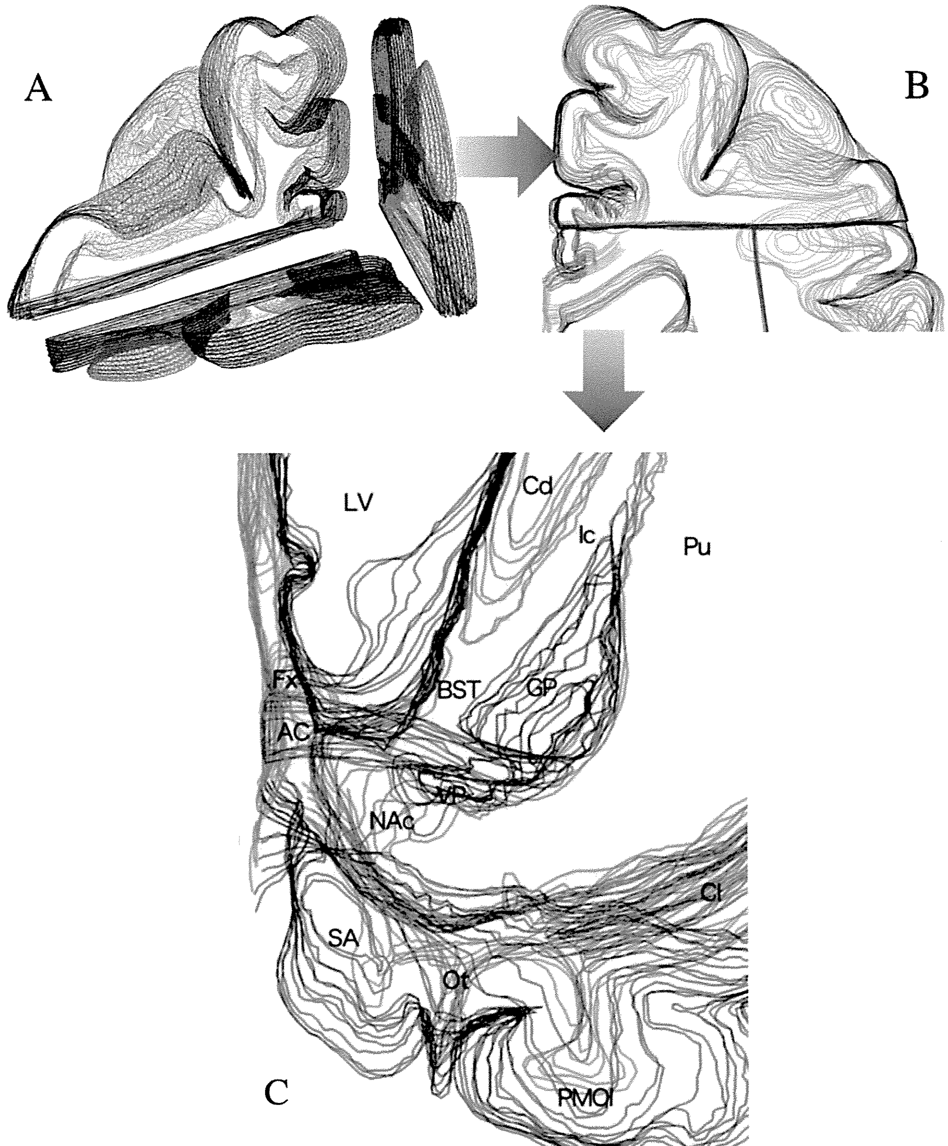

Fig.2 The contours of serial histological microslices were successfully reconstructed (A). The blocks could be put side by side and assembled into large block without any correction or revision (B). Contours of anterior basal ganglia could be clearly demonstrated according to cyto-architecture (C). (LV, lateral ventricle; Cd, caudate nucleus; Ic, internal capsule; Pu, putamen; Fx fornix; Ac, anterior commissure; BST, bed nucleus of stria terminalis; GP, globus pallidus; VP, ventral pallidum; NAc, nucleus accumbens; SA, subcallosal area; Ot, olfactory tract; Cl, claustrum; PMOl, posteromedial orbital lobule)

結　果

非接触型三次元デジタイザの空間分解能は0.456 mmである。一方，最新のMRIやCTでも0.5 mm厚の医用画像を取得できるが，この時の三次元空間分解能は1.12 mmである。またMRIやCTではsegmentationの閾値設定により脳の形状はいくらでも変化する。これらを比較すると，デジタイザで得られる形状データは最新の医用画像より密で高精度であることがわかる。カメラから観測可能な物体表面のみの形状データが得られるが（Fig.1-B），対象物の姿勢を変えながら異なる視線方向から物体の全周表面を計測し，全計測データを貼りあわせることで全周表面形状モデルの生成を試みた。具体的に，各計測データはそのデータを取得した視線方向に依存した座標系で記述されている。これらの座標系の位置合わせを，ICP (Iterative Closest Point) アルゴリズ

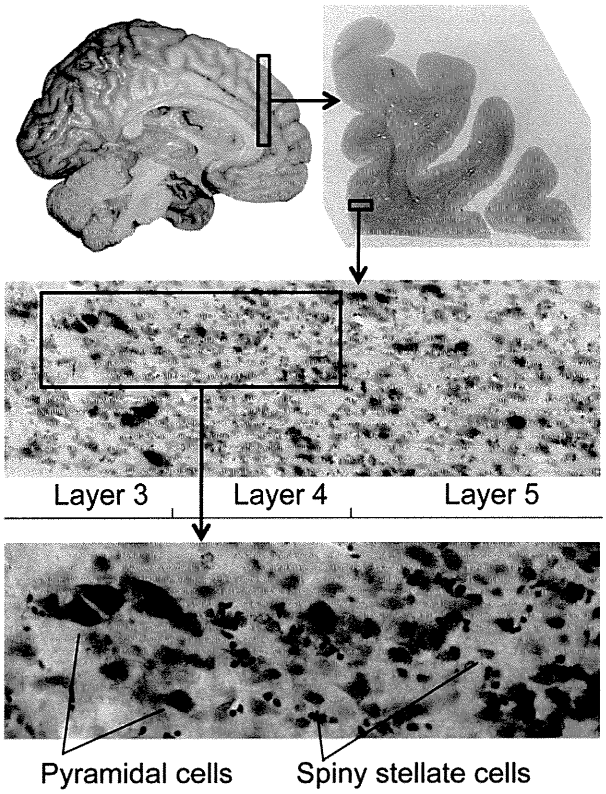

Fig.3 Nissl-staining of the section used for brain atlas reconstruction. Histological sections included in this partially reconstructed brain atlas provided the excellent preservation of area-specific cytoarchitecture of cerebral cortex; Brodmann area 32 was discriminated by the presence of large pyramidal neurons in deep layer III and spiny stellate neuron in layer IV in cadaver brain.

ムを用いて行い，その結果から全計測データを統一した世界座標系に変換する。ICPアルゴリズムは，ある物体表面領域のデータを異なる方向から取得し，その領域内の対応関係に基づいて位置合わせを行う。そのため冗長なデータが存在しているため，このようなデータを削除するとともに，表面再構築を行わなければならない。Moorokaらが提案した手法にもとづきデータ統合と表面再構築を行い[5]，最終的に非常に滑らかな表面形状を復元できた（Fig.1-C)。

脳ブロックの一つから得た再構築像を異なる方向から示す（Fig.2)。レンダリング等の改変は加えていない。ヒト大脳皮質に特有の深い脳溝や複雑な脳回が正確に再現されている（Fig.2-A)。3つのブロックから得たトレース像をそれぞれ拡大縮小や変形することなく限りなく近づけ，一つの像に組み合わせると，ブロック断面で矛盾はなく高い整合性が得られた(Fig.2-B)。このブロック内部の様々な領域はトレースの過程で組織の顕微鏡観察により同定できた(Fig.2-C)。

さらに再構築に用いた献体脳の帯状回Brodmann area 32の光顕像(ニッスル染色，Fig.3)では，第Ⅲ層の大錐体細胞や第Ⅳ層の有棘星状細胞が明瞭に確認され，大脳皮質層構造に特異的な細胞構築がよく保たれていることがわかった。

考　　察

機能神経外科手術においてヒト脳アトラスは，Schaltenbrand & Wahrenアトラス[13]が最も広く応用されており，現在のような最先端医療におけるコンピュータ技術の貢献を予見し，Yoshidaは初めて大脳アトラスの電子化を試みた[16]。現在ではTalairachグリッド[14]で3軸の単純変倍操作を加えながら[10]，患者のMRI画像に適合できるよう，殆どの定位機能神経外科手術支援ソフトウェア（SurgiPlan, FrameLink, StereoPlan, Cerefyなど）に搭載されている。しかし実際の脳形状は個人差に富み，Talairachグリッドで補正できるほど容易ではないため[8]，機能的定位脳手術においては侵襲的な微小電極記録で標的神経核を電気生理学的に確認する作業が求められる。しかしヒト脳の場合，電気生理学的研究の対象は不随意運動や難治性疼痛などの機能的脳疾患の特定の神経核に限られており，それら以外の神経核・白質の電気生理学的知見は殆どない。個体差に対応できる正確なアトラスであれば，微小電極記録という侵襲的操作を必要せず，新規の神経核を標的とした機能的定位脳手術が期待できるが，そのためには複数体から得られる平均的・普遍的な解剖情報(座標)が必要になる。

また脳のサイズや形状には明らかな人種差があり，東アジア人の脳や神経頭蓋は西洋人に比較して前後径が短く幅が広く球体に近い形状となっている[12]。脳内構造の人種差は全く未知の領域であるが，少なくとも西洋人単一個体の脳を普遍化するのは無理で，ましてや単純な変倍操作によって日本人の脳内座標を決めるのは非常に危険である。この不正確さを補うためには侵襲的な電気生理学的検索が必要であるが，それでも精度には限界があり脳内出血など合併症のリスクがつきまとうことになる。

病理検査や外科治療の際に得られる知見を除き，本邦ではヒト脳が解剖学的研究材料として扱われることはほとんどない。ヒト脳のように柔らかく大きな臓器を変形させせることなく切片化し，なおかつ良質の組織像を得るためには，セロイジン樹脂（現在入手不能）固定を要するが，樹脂が組織全体に浸透するのは膨大な時間（数ヵ月～1年がかり）を要する作業であり，さらに大型ミクロトームなど特殊な機材・環境と熟練を要する。そのため大脳半球全体ではなく，視床や基底核・脳幹部など深部構造に限られた狭い範囲で凍結標本を作成し精密に座標化するか[1,3,4]，ある程度の機械的変形や微細細胞構築の破壊を無視して脳半球全体の凍結標本で座標化するかの選択に迫られる。Yelnik ら[15]は，死後のMRIで脳形状を記録し，作成した凍結固定スライス標本を検証し補正に用いた。しかし固定処理前に頭蓋から摘出されたため重力の影響で凍結前に変形しており，作製されたスライス標本では脳室が潰れ肉眼的に大きく変形している。また表面部（脳表や脳室壁）は凍結固定に伴う組織損傷が著しい。肉眼的変形は摘出前MRIを指標とした補正処理を行えば，一応，基底核や視床に限局したアトラスは作製可能である。しかし微細な細胞構築が保たれていないため亜核構造などの同定は不可能である。このような先行研究からも，ヒト大脳という巨大で柔らかい組織を変形なく細胞構築を温存し全体を連続標本にすることがいかに困難なのかがわかる。

我々の開発した方法では，脳は頭蓋から取り出す前に（*in situ*）十分ホルマリン固定されている上，三次元デジタイザで正確に脳表の形状保存ができ，agarで周囲を支持することでブロック化に伴う肉眼的変形を防ぎ，振動刃マイクロスライサで細胞構築を保ちつつスライスすることにより，三次元的整合性と美しい組織染色画像を両立できることがわかった[2]。

ヒト脳アトラス作成の第一段階として，大脳全体を用いてより普遍的な形状モデルを得ることが目標である。将来の機能的定位脳手術において，様々な機能的脳疾患に対し，様々な治療標的（神経核や神経線維）が開発される可能性を想定すると，皮質や脳幹も含めた広範囲で，三次元的に自由に形状を調整できる次世代型座標アトラスを作成することが望まれる。

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文 献

1) Chakravarty MM et al: The creation of a brain atlas for image guided neurosurgery using serial histological data. NeuroImage 30: 359-376, 2006.

2) Fukuda T et al: A simple but accurate method for histological reconstruction of the large-sized brain tissue of the human that is applicable to construction of digitized brain database. Neurosci Res 67: 260-265, 2010.

3) Hirai T et al: Distribution of tachykinin- and enkephalin-immunoreactive fibers in the human thalamus. Brain Res Rev 14: 35-52, 1989.

4) Morel A et al: Multiarchitectonic and stereotactic atlas of the human thalamus. J Comp Neurol 387: 588-630, 1997.

5) Morooka K et al: Self-organizing Deformable Model : A new method for fitting mesh model to given object surface. International Symposium on Visual Computing, LNCS 3804, pp.151-158, 2005.

6) Niemann K et al: Verification of the Schaltenbrand and Wahren stereotaxic atlas. Acta Neurochir (Wien) 129: 72-81, 1994.

7) Niemann K et al: One atlas- three anatomies: Relationships of the Schaltenbrand and Wahren microscopic data. Acta Neurochir (Wien) 141: 1025-1038, 1999.

8) Niemann K et al: A brainstem stereotactic atlas in a three-dimensional magnetic resonance imaging navigation system: first experiences with atlas-to-patient registration. J Neurosurg 90: 891-901, 1999.

9) Niemann K et al: The Morel stereotactic atlas of the human thalamus: Atlas-to-MR registration of internally consistent canonical model. NeuroImage 12: 601-616, 2000.

10) Nowinski WL: Anatomical targeting in functional neuro-surgery by the simultaneous use of multiple Schaltenbrand-Wahren brain atlas microseries. Stereotact Funct Neurosurg 71: 103-116, 1998.

11) Nowinski WL et al: The Cerefy Clinical Brain Atlas on CD-ROM. New York, Thieme, 2004.

12) Rushton JP et al: Brain size, IQ, and racial-group differences: Evidence from musculoskeletal traits. Intelligence 31: 139-155, 2003.

13) Schaltenbrand G et al: Atlas for stereotaxy of the human brain. Thieme, Stuttgart, 1977.

14) Talairach J et al: Co-planner stereotactic atlas of the human brain. Thieme, New York, 1988.

15) Yelnik J et al: A three-dimensional, histological and deformable atlas of the human basal ganglia I. Atlas construction based on immunohistochemical and MRI data. NeuroImage 34: 618-638, 2007.

16) Yoshida M: Creation of a three-dimensional atlas by interpolation from Schaltenbrand- Bailey's atlas. Appl Neurophysiol 50: 45-48, 1987.

Application of digital imaging technique for the construction of stereotactic human brain atlas

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Abstract: An ideal human brain atlas must provide the universal coordinates of standard healthy brain. Morphology of human brain contains considerable inter-individual variety; however, the classical human brain atlases have been made from a limited number of materials. A large organ such as human brain is subject to mechanical deformation and the production of stereotactic human brain atlases is still time-consuming and needs special instruments, environments and skills. To achieve both the spatial consistency and high histological quality for stereotactic human brain atlases, we have established a novel technique for constructing the stereotactic brain atlas using a formalin-fixed cadaver brain of the Japanese, blade-oscillation microslicing and digital imaging techniques. Our method enabled the accurate reconstruction of human brain histological slices with the three-dimensional consistency necessary for the stereotactic atlases of human brain, as well as the successful preservation of original macroscopic shape and cytoarchitecture.

Keywords: Human brain atlas; Histological section; Stereotactic functional neurosurgery

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ジストニア・パーキンソン病の定位脳手術支援のための脳座標アトラス

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Digital brain atlas for the stereotactic neurosurgery of Dystonia and Parkinson's disease

Yasushi Miyagi, Ken-ichi Morooka, Takaichi Fukuda, Akio Kuraoka, Kenji Sunagawa, Tsuyoshi Okamoto, Takashi Yoshiura, Xian Chen, Taketo Hayami, Shozo Tobimatsu

1. 緒言

ジストニアやパーキンソン病など不随意運動に対する機能的定位脳手術では、精密なヒト脳アトラス[1)]を参考に第3脳室の交連間線と Talairach グリッドで補正を加えた上で手術部位が計画される。しかし実際の脳形状は個人差に富むため、侵襲的な微小電極記録で標的神経核を確認する作業が求められる。特に現在の脳アトラスは欧米人脳から作成されているため、我が国の機能的定位脳手術において日本人に特化した脳アトラスが求められる。我々は工学的手法を応用し、ヒト大脳アトラスの開発に着手した。我々の理想とする脳アトラスは日本人のホルマリン脳を材料とし、三次元的整合性を持ち、脳形状の個体差に対応して自由に変形に可能でき、年齢や脳疾患による部位依存性萎縮要素を加味し、複数の位置情報と機能情報を統合し格納・更新できるデジタルアトラスである。

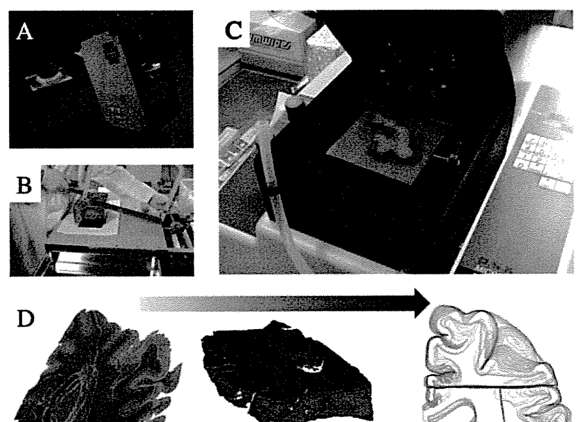
2. 対象と実験方法

日本人脳は正常解剖実習用のホルマリン固定脳を用いた。非接触型3次元デジタイザーを用い、標本作製に伴う変形・欠損を補正するために脳全体の初期形状を記録した(Fig.1A)。まず 3% agar に包埋した脳を正確に平行切断し 1cm 厚にブロック化する方法を開発した(Fig.1B)。切断後にいったん agar を除去し、再度、非接触型3次元デジタイザーで各ブロックの形状を記録した。次にブロックをゼラチンに包埋後、全体を 4%パラフォルムアルデヒドで一晩固定し、大型の振動刃ミクロトームを用いて組織学標本用の連続切片を作成した(Fig.1C)。また包埋したゼラチンの外枠部分4ヶ所に垂直に針を刺入しマーキングすることで連続切片から3次元再構築する際のリファレンスを得た(Fig.1D)。

3. 結果

最終的な切片標本において、マーキング間の長さを測定し、変動係数(coefficient of variance)が 0.005 と標本作製に伴う変形がごくわずかであることが分かった。

Figure 1



4. 今後の展開

今回確立した大型組織標本作製法で一体分の日本人脳の完全な連続標本を作成し電子化する（デジタルアトラス）。また第二段階として複数体の脳 MRI 画像から得た脳形状デジタルデータを平均化し、より標準的な形状を作成する（ハイブリッドアトラス）。また第三段階（デフォーマブルアトラス）として、標準脳形状を各ジストニアやパーキンソン病患者の MRI 画像に対応し三次元的に自由に変形できるアルゴリズムを開発し、第四段階として淡蒼球内節や視床下核など手術標的を正確に提示できるテイラーメイドアトラスとして検証する。第五段階で脳内座標に対し機能情報を格納し、文献検索やデータマイニングのできるデータベースアトラス、開頭手術計画や手術訓練に用いるシミュレータアトラスへ発展させたい。

参考文献

1) Schaltenbrand G, Wahren W: Atlas for stereotaxy of the human brain. Thieme, Stuttgart, 1977

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Technical note

A simple but accurate method for histological reconstruction of the large-sized brain tissue of the human that is applicable to construction of digitized brain database

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ABSTRACT

Research on the human brain has undoubted significance, but our knowledge on its detailed morphology is still limited. We have developed a simple method for reconstruction of large-sized brain tissues of the human. Fixed brains were cut into blocks (maximum size 7 cm × 7 cm × 1 cm), embedded and post-fixed in gelatin just one overnight before obtaining complete serial sections with a vibrating microtome. Quality of stained materials was sufficient to create three-dimensional histological maps, where digital reconstructions from adjoining blocks could be accurately combined. The present method will facilitate both direct examination of the human brain and construction of its histological database.

1. Introduction

Most neuroscientists in any research field would agree that one of the ultimate goals of our study is to know the human brain even though we do not directly handle it. Animal experiments are thus designed to address various issues through the sophisticated methods that are difficult to conduct in humans, and the obtained results are expected to be applied to the human nervous system. However, structures of the brain show substantial interspecies differences such that even non-human primates have the brains that are different from ours (Foxman et al., 1986; Caminiti et al., 2009). Therefore some cautiousness is necessary when one interprets animal data in relation to human brain functions in either normal or pathological condition. In this context there is a critical problem that quantity of our knowledge about brain anatomy shows profound differences between humans and experimental animals. The vast majority of modern neuroanatomical studies have been executed in animals, whereas structural details in many anatomical regions of the human brain still remain in the range of what were described in classical studies. Therefore more investigations are necessary to illuminate the internal architecture of the human brain in light of the recent findings in other fields. The rapid progress in functional imaging studies particularly requires updating our knowledge on human brain anatomy.

One practical reason for the difficulty in investigating human brains would be their extraordinary large size. For an in-depth analysis of a particular anatomical region, observations of serial sections covering its entire field as well as its surround are essential. However, for that purpose a huge microtome and exceptionally large slides must be prepared, an extremely long period is necessary for dehydrating and embedding large brain blocks, and the proficiency in craft works of a rather old-fashioned style is another requirement. All these conditions are incompatible with modern laboratories where mouse brains are most frequently used. Although a few big projects aiming at preparing serial frozen sections from the whole cadaver such as Visible Human Project are in progress, a large amount of funds as well as specially made machineries are prerequisite. These circumstances would lead to few opportunities of detailed examination of human brain structures despite the availability of the materials; most traditional medical schools would have the donation program for cadavers used in their education of gross anatomy. We therefore intended to develop a simple method that enables researchers in conventional anatomical laboratories to prepare and analyze complete serial sections of the human brain covering large region of the brain. The obtained histological specimens had sufficient quality to construct image database of the human brain at the light microscopic level that cannot be prepared by radiological methods.

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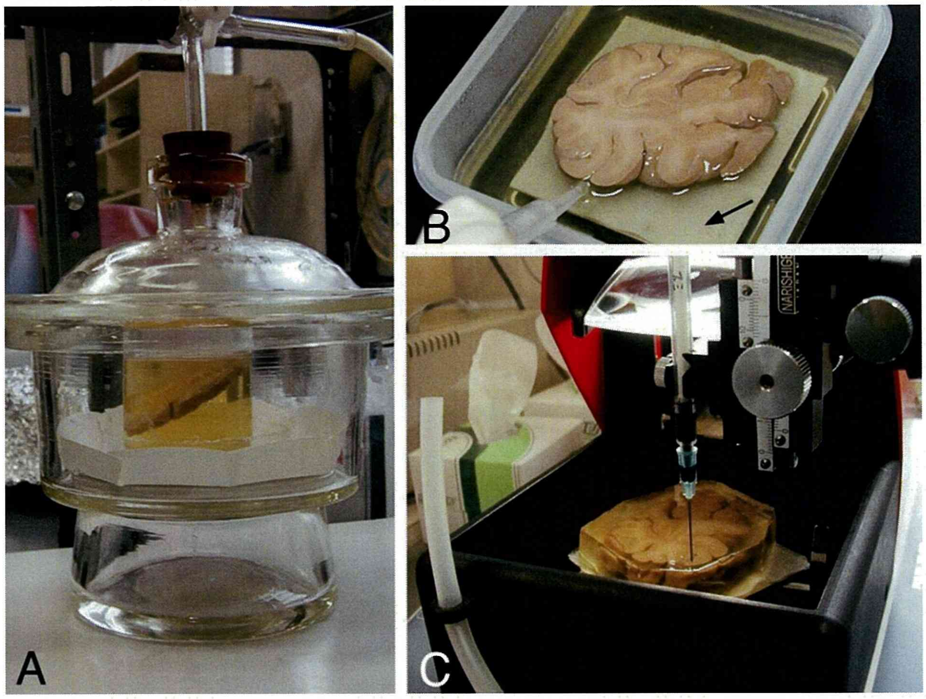

Fig. 1. Embedding of the brain block into gelatin. (A) The block is soaked in gelatin solution and lightly depressurized to replace the air trapped in the deep part of the tissue with gelatin. (B) The brain block is laid on a 3 mm-thick layer of gelatin that was hardened and immobilized beforehand through the MAGICTAPE® (arrow) stuck on the metal stage, then gelatin solution is poured over the whole block. (C) The reference tracks are made into the surrounding gelatin by applying India ink through the vertical needle.

2. Materials and methods

2.1. Subject

A brain of a cadaver, 89-year-old male Japanese, was used in the present study. The cadaver was donated to Kyushu University Faculty of Medicine by the donation scheme of cadavers, 'Shiragiku-Kai (White Chrysanthemum Society)', that includes the understanding and written consent of each member of the Society. The documented acceptance of the storage of histological materials was further obtained from the bereaved family. The conventional procedures of preparing materials for gross anatomy practical course were performed. Thus 5 litters of 10% formalin were injected through the femoral artery, the body was left untouched for 12 h, then immersed in an alcohol solution (Solmix H-11) for several weeks. Thereafter the body was stored at 4 °C before use in the practical course for medical students. The brain was removed during the course and stored at 4 °C in phosphate-buffered saline containing 0.1% sodium azide. After removing the arachnoid membrane the whole surface of the brain was scanned by a non-contact 3D digitizer (VIVID910, Konica Minolta, Sakai, Japan). By this method the shape of the brain was accurately measured and digitally stored before histological preparations. Subsequently the brain was sagittally cut into two halves and each hemisphere was embedded in 10% agar. Using the specially designed brain-cutting tool (Visceracut VC-600, Meiko Medical, Munakata, Japan), the brain embedded in agar was cut into serial coronal slices, each 1 cm in thickness.

2.2. Embedding procedures

The surrounding agar and the remaining membranous tissues and blood vessels on the surface of the slice were carefully removed under operation microscope. As the maximum size of histological sections available in the present method was 7 cm × 7 cm, each slice was further divided into 2 or 3 blocks when necessary. The block was soaked in 10% gelatin (~40 °C) and lightly depressurized to replace the air trapped in the deep part of the tissue with gelatin (Fig. 1A). In order to cut complete serial sections from the whole block, tissues were embedded in gelatin as follows (Fig. 1B): adhesive tapes with hook and loop fasteners (MAGICTAPE®; Kuraray, Osaka, Japan) were applied on the metal surface of the sample stage (7 cm × 7 cm) of the microtome, a 3 mm-thick layer of gelatin was poured and hardened on the MAGICTAPE®, tissue block was laid on it, then gelatin solution was again poured over the tissue block. The entire tissue was thus put inside the gelatin that was made immobile on the sample stage, and the serial sectioning was continued until tissues at the bottom surface of the block were collected. To make reference tracks for reconstructions a 23G needle attached to a tuberculin syringe was vertically penetrated into the surrounding gelatin (but not inside the tissue) at 4 points using stereotaxis manipulator (Narishige, Tokyo, Japan) and applying India ink. The gelatin block was further hardened by fixation with 4% paraformaldehyde in phosphate-buffered saline overnight before sectioning.

2.3. Histology

Serial sections were cut with a vibrating microtome (DTK-3000W, Dosaka, Kyoto, Japan) at 100 μm thickness. Each fourth section was mounted on a slide coated with gelatin and chromium alum, dried and processed for Nissl staining using thionin. To improve staining quality, the following procedure that removes lipid by mimicking paraffin embedding was effective (Fukuda et al., 1993): sections were dehydrated with ascending ethanol, cleared in xylene, then rehydrated before

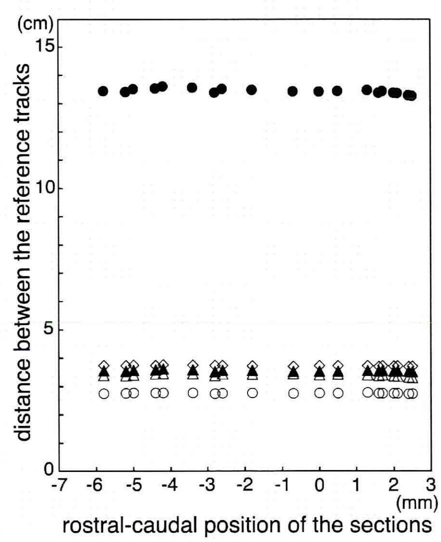

Fig. 2. Deformity-free preparation of the serial sections. The distance between the reference tracks was measured in individual sections and compared along the rostral-caudal position of the sections. Both the distances between each pair of the 4 tracks (bottom) and the sum (perimeter of the square; top) are kept constant throughout the serial sections.

use in Nissl staining. Neighboring sections were treated with 0.2% osmium tetroxide to visualize myelinated structures that facilitated identification of the tissue architecture.

2.4. Analysis

Images of stained materials were digitized by a scanner (GT-X970, SEIKO EPSON, Nagano, Japan) attached to a personal computer (NetVista, IBM Japan, Tokyo, Japan) and stored in TIFF format. The distance between the reference tracks in each section was measured by an application ImageJ (NIH). The tissue architecture was reconstructed three-dimensionally by tracing the contours of different anatomical structures inside individual sections with a computer-assisted neuron tracing system (Neurolucida, MicroBrightField, Williston, USA). Reference tracks were used to align consecutive sections. Three-dimensional view of the reconstructed materials was made possible by the Neuroexplorer Solid modeling module (MicroBrightField).

3. Results

3.1. Preparation of complete serial sections in a short period with the least deformity

Embedding tissues in gelatin and cutting them with a vibrating microtome obviated several difficulties in preparation of histological materials from the human brain. It took just one overnight to embed a large block before cutting, and complete serial sections could be obtained on the following day. This is contrasted with the conventional paraffin or celloidin preparations that require long dehydration procedures lasting weeks to months for large blocks.

The combination of gelatin embedding and use of a vibrating microtome also had advantage in accurate sectioning. Dehydra-

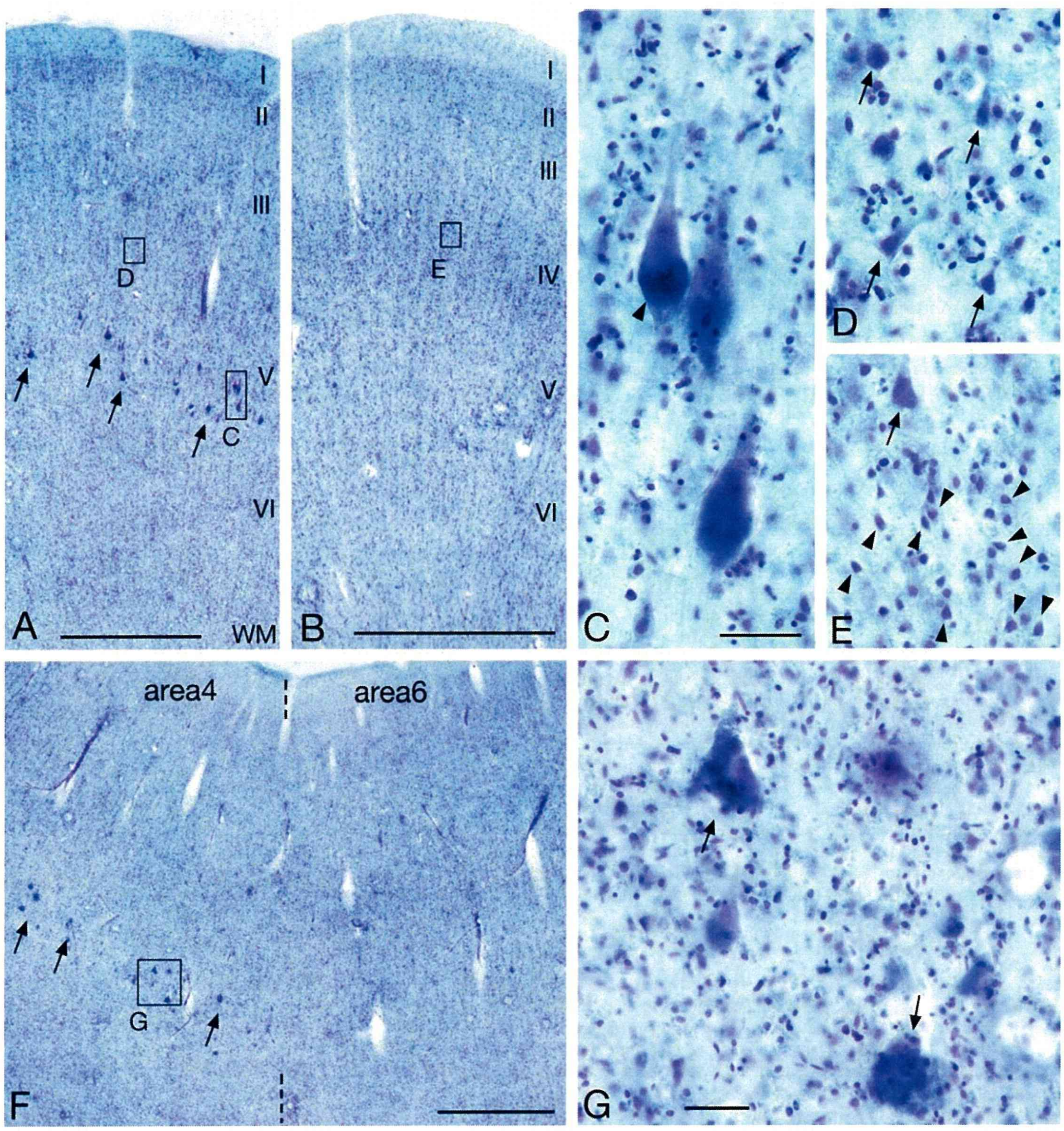

Fig. 3. Light micrographs showing the quality of the staining. (A) Low-power view of the cortical area 4 of Brodmann located in the dorsal part of the precentral gyrus. The position of the tissue inside the original brain is shown by a yellow triangle in Fig. 6. Giant pyramidal cells of Betz (arrows) are seen in layer V, some of which are enlarged in C. The more superficial region around the border to layer III (left box) is shown in D. (B) The postcentral gyrus characterized by the presence of well-developed layer IV, part of which is enlarged in E. (C) Enlargement of the giant pyramidal cells of Betz shown in (A). Lipofuscin granules (arrowhead) are highly accumulated inside the cells. (D) Pyramidal cells of medium to relatively large size are shown by arrows. (E) Enlargement of the upper part of layer IV in B. Arrowheads indicate small, presumptive spiny stellate cells. A pyramidal cell of a large size is located at the bottom of layer III (arrow). (F) The border between area 4 and area 6. Giant pyramidal cells of Betz (arrows) in area 4 disappear abruptly at the border toward the neighboring area6 where agranular laminar pattern is similar to that in area 4. (G) Enlargement of giant pyramidal cells (arrows) in a boxed region in F. Scale bars: 1 mm (A, B, F); 50 μm (C–E, G) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).

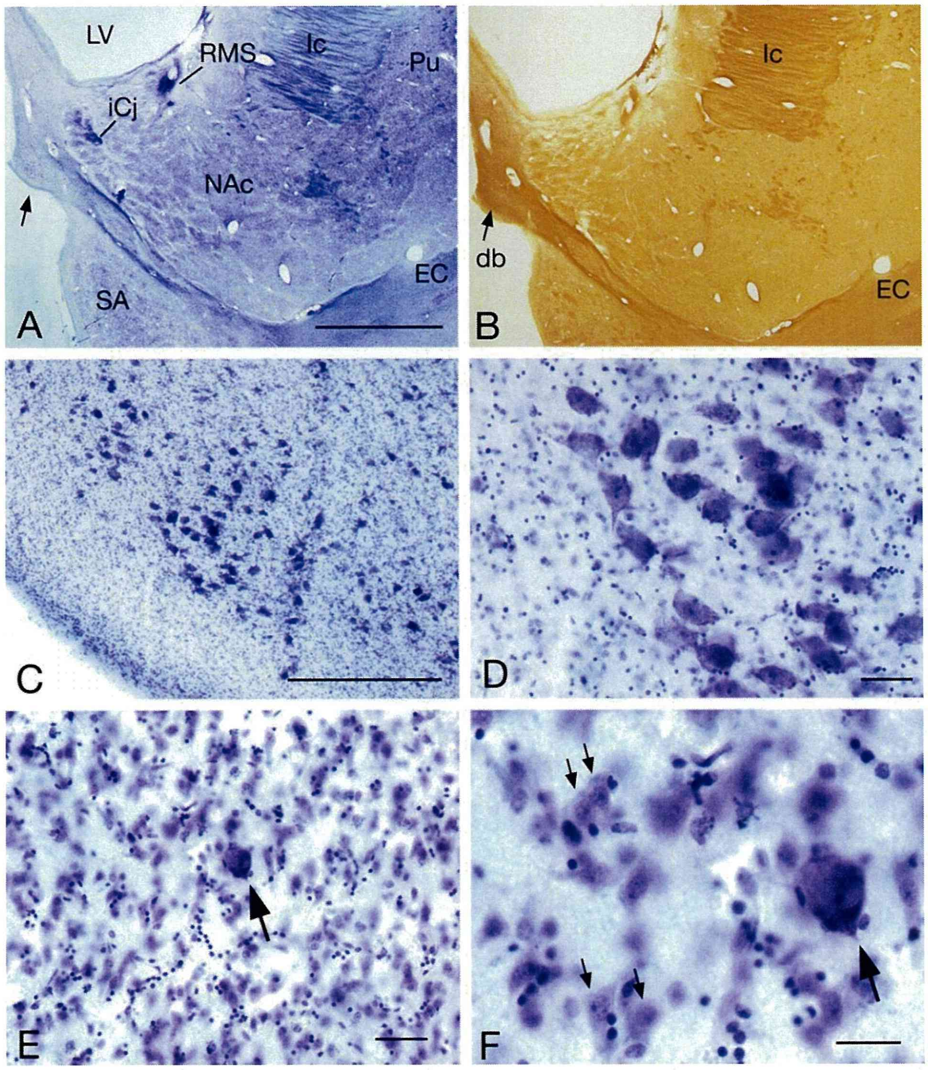

Fig. 4. Light micrographs showing the staining quality in subcortical structures. (A) Low-power view of the region around the accumbens nucleus (NAc). Other structures are: iCj, islands of Calleja; RMS, rostratory migratory stream that could be traced in serial sections to the olfactory tract; LV, lateral ventricle; Ic, internal capsule; Pu, putamen; SA, subcallosal area; EC, external capsule. The arrow at the left side indicates the nucleus of diagonal band that is enlarged in C and D. (B) Myeloarchitecture in the neighboring section stained with osmium tetroxide. Arrow indicates the diagonal band (db). (C and D) The nucleus of diagonal band contains numerous large cells. (E and F) Enlargement of the putamen characterized by medium-sized neurons (small arrows in F) and a much larger, presumptive cholinergic giant aspiny cell (large arrows). Scale bars = 5 mm (A and B); 500 μm (C); 50 μm (D and E); 100 μm (F).

tion before the conventional embedding procedures unavoidably causes shrinkage of the block and the resultant deformity due to the anisotropy in shrinkage. Gelatin embedding in the present method could skip this process. Moreover, as shown in Fig. 2 it also minimized the deformity after cutting. The distances between reference tracks were measured in each section, and the coefficient of variation (standard deviation/mean) was calculated in individual blocks. The distances were found to be kept constant throughout the series; the coefficient was at quite low levels (0.005–0.01, mean 0.008; $n = 10$ blocks).

3.2. 3D reconstruction from serial sections

Thickness of sections was optimized to 100 μm in order to obtain serial preparations. This relatively large thickness did not affect observations of the cytoarchitecture that is unique to and indispensable for the identification of each anatomical region. For example, specific laminar patterns of various neocortical areas could be distinguished (Fig. 3), and the presence of characteristic neuron populations was recognizable in different nuclei (Fig. 4).

Based on the accuracy and quality of the tissue preparation, structures contained in serial sections were traced for 3D-reconstructions by using the computer-assisted system (Fig. 5). As seen in Fig. 5A, the reconstructions had smooth surfaces without any corrections of the traced data. When 1 cm-thick slice had been cut into 2–3 blocks before microtome sectioning, reconstructions from these blocks could be put together to recover the original 1-cm thick slice without the necessity of scaling (Fig. 5B, Supplementary Fig. 1). As the data were stored in a digital format, the complicated 3D morphology of the internal structures such as basal ganglia (Fig. 5C) was observable in different viewing angles (Fig. 6). The 3D view of the recovered slice was then compared with both the non-contact 3D digitizer model and the photograph of the hemisphere to know its original position within the brain. Correspondence of gyri and sulci between the reconstructions and the original brain was recognizable as in Fig. 6. As the digital files for reconstructions are composed of numer-

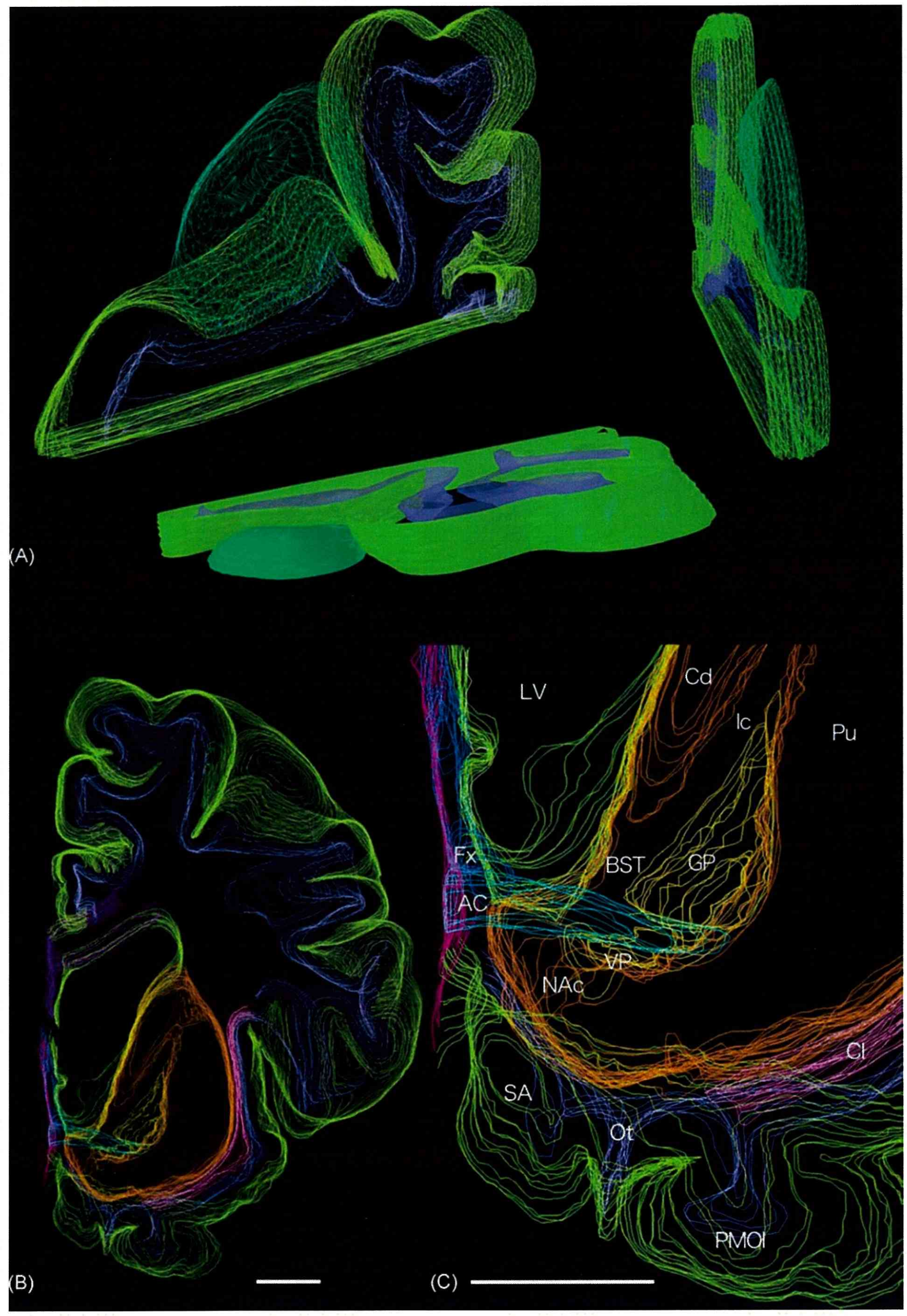

Fig. 5. Reconstruction from the serial sections. (A) Computer-assisted tracings of a brain block near the central sulcus are reconstructed and viewed from different angles. Note smooth surfaces of the reconstructed images that are free from correction of the original tracing data. (B) A composite image of the three reconstructions (Supplementary Fig. 1) that are put together without scaling. (C) Enlargement of a part of (B), indicating various internal architecture: AC, anterior commissure; BST, bed nucleus of stria terminalis; Cd, caudate nucleus; Cl, claustrum; Fx, fornix; GP, globus pallidus; Ot, olfactory tract; PMOl, posteromedial orbital lobule; VP, ventral pallidum. Other abbreviations as Fig. 4. Scale bars: 1 cm.

ous spatial points having 3D coordinates, calculation of the best fit between these data and the coordinates within the 3D digitizer model is possible, which is now in progress (Morooka et al., 2009).

4. Discussion

The present study has established a simple method for preparing serial sections of large-sized brain tissues of the human. Combination of gelatin embedding and use of a vibrating microtome has led to both shortening of time for tissue preparation and minimizing the deformity of the specimens. The obtained sections were processed for Nissl staining that is the standard method for identifying tissue structure of the brain, for which the present materials had enough quality. Moreover, even such a simple staining method has revealed unique tissue architectures in the basal forebrain that was quite different from those in rodents (our unpublished observations).

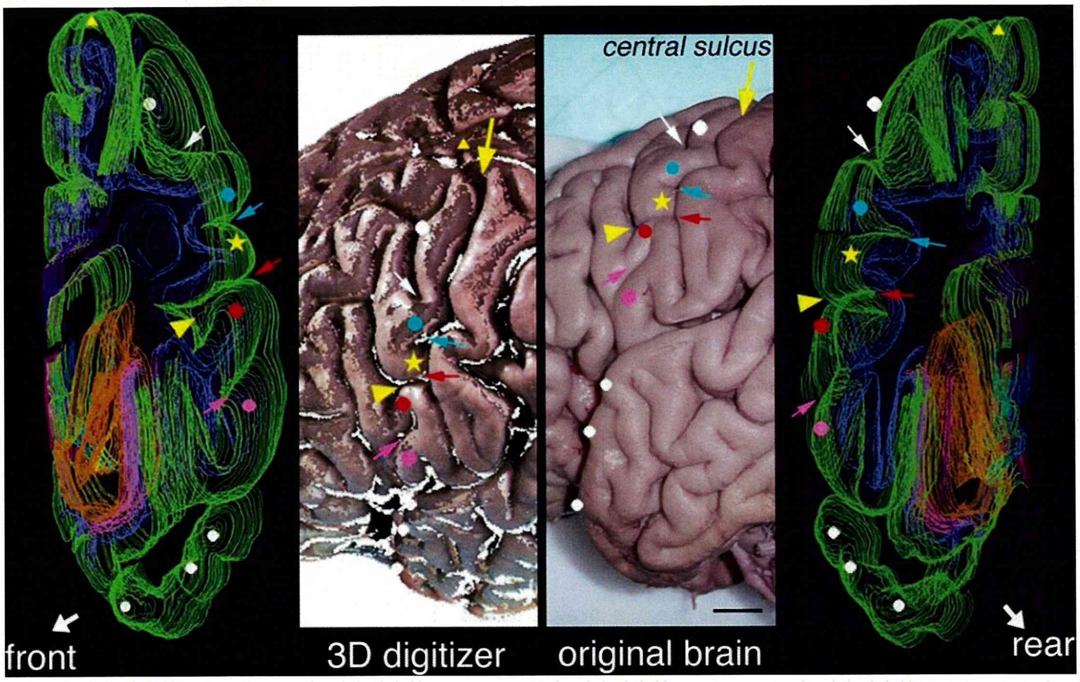

Fig. 6. Correspondence of the reconstructions and 3D digitizer model with the original brain. The same sulci and gyri are shown by the same arrows and symbols, respectively. Yellow large arrows indicate the central sulcus. Reconstructions shown on both sides of the figure are the images viewed from different angles. Yellow triangles on top of the reconstructions are located in the dorsal part of the precentral gyrus and correspond to the site of light micrograph in Fig. 3A. Scale bar: 1 cm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The material used was not processed systematically for immunohistochemistry, as the conventional procedures for gross anatomy course at our institute are not necessarily appropriate for it. However, specific immunostaining was obtained for methionin-enkephalin in our preliminary study on a large specimen containing both the dorsal and ventral striatum. The present method will be applicable to immunohistochemical examinations of various neuroactive substances as far as the condition of the tissue is well preserved, and in that case immunohistochemistry will be possible over the large-sized nuclei of the human brain.

There might be some arguments on the possible postmortem change in the gross morphology of the brain. Deformity of this type will more or less happen in conventional autopsy cases, because in pathological examinations the untreated, soft and fragile brain is removed from the skull and is fixed thereafter by immersing it in a solution for a long period. By contrast, the postmortem deformity in the present method is thought to be minimum, as the structure of the whole body was preserved by perfusion of the fixative from the femoral artery and tissues were thus hardened with formalin *in situ*. As a result the gross morphology of the whole cadaver including the brain was well preserved when dissection.

Obtained materials can be scanned and stored as digital image files at the light microscopic level that can never be provided by CT or MRI data. The image database can be used for three-dimensional reconstructions of the human brain and further for development of histological reference maps applicable to modern neurosurgical treatments. The deep brain stimulation in particular is now targeting more confined regions for broader disease spectrum than currently executed (Miyagi et al., 2009), thus requiring more precise knowledge on finer anatomy (Miyagi et al., 2007). The present method will be able to provide the database for reconstructing the digitized brain of the human that can be applied to brains of individual patients by using the best algorism of non-rigid deformation (Morooka et al., 2007).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neures.2010.03.005.

References

Caminiti, R., Ghaziri, H., Galuske, R., Hof, P.R., Innocenti, G.M., 2009. Evolution amplified processing with temporally dispersed slow neuronal connectivity in primates. Proc. Natl. Acad. Sci. U.S.A. 106, 19551–19556.

Foxman, B.T., Oppenheim, J., Petito, C.K., Gazzaniga, M.S., 1986. Proportional anterior commissure area in humans and monkeys. Neurology 36, 1513–1517.

Fukuda, T., Nakano, S., Yoshiya, I., Hashimoto, P.H., 1993. Persistent degenerative state of non-pyramidal neurons in the CA1 region of the gerbil hippocampus following transient forebrain ischemia. Neuroscience 53, 23–38.

Miyagi, Y., Shima, F., Sasaki, T., 2007. Brain shift: an error factor during implantation of deep brain stimulation electrodes. J. Neurosurg. 107, 989–997.

Miyagi, Y., Okamoto, T., Tobimatsu, S., Nakanishi, Y., Aihara, K., Hashiguchi, K., Murakami, N., Yoshida, F., Samura, K., Nagata, S., Sasaki, T., 2009. Spectral analysis of field potential recordings by deep brain stimulation electrode for localization of subthalamic nucleus in patients of Parkinson's syndrome. Stereotac. Func. Neurosurg. 87, 211–218.

Morooka, K., Matsui, S., Nagahashi, H., 2007. Self-organizing deformable model for mapping 3D object onto arbitrary target surface. In: Proceedings of the 6th International Conference on 3-D Digital Imaging and Modeling, pp. 193–200.

Morooka, K., Miyagi, Y., Fukuda, T., Okamoto, T., Hayami, T., Chen, X., Sunagawa, K., Tobimatsu, S., Yoshiura, T., Hashizume, M., 2009. Digital brain atlas for safe and accurate stereotactic neurosurgery. In: Abstract in 6th Annual World Congress for Brain Mapping and Image Guided Therapy.

A02-9 Construction of Brain Simulator System for Computer-aided Diagnosis and Therapy: Progress Overview FY2010

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***Abstract*—The purpose of this paper is to construct a brain simulator for the establishment of safe and accurate functional neurosurgery. Classical human brain atlases lack in universality and 3D-consistancy because of limited number of human brain materials. Using the digital imaging technique and micro-slicing technique, we have developed a novel method to construct the stereotactic brain atlas of human with the successful preservation of both histological quality and stereotactic accuracy. This method enables the stereotactic brain atlas of the specific populations, such as race and disease, and enables tailor-made neurosurgery. Japanese specialized for functional neurosurgery. In this project, we are going to extend the stereotactic brain atlas into the universal and multipurpose brain simulator. The future role of this brain simulator is to work as a knowledge database in relation to the local structure of the human brain and as a core of computational anatomy in neuroscience.**

I. BACKGROUND

The increase in stereotactic and functional neurosurgery in Japan has urged us to develop an ideal human brain atlas, especially specified to the Japanese. An ideal brain atlas must provide the universal coordinates of standard healthy brain, however, the morphology of human brain inevitably contains considerable inter-individual variety while the classical human brain atlases have been made from limited number of brain materials [1], [2]. Only a simple magnification in each axis is a rationale to apply an atlas to individual patient (Fig.1), apart from tailor-made surgery. Probabilistic functional atlas [3] includes a postoperative surgical result of large population and probable distributions of only three functional targets (internal segment of globus pallidus, subthalamic nucleus and nucleus ventrointermedius of thalamus), which may suggest a tentative coordinates for stereotactic functional neurosurgery. Actually, however, what we need is a clear boundary of functional target structure but not probability in individual clinical practice. The tailor-made neurosurgery can be achieved in a true sense, only when the atlas could be deformed to fit to the individual brain. Furthermore, stereotactic functional neurosurgery is increasingly being introduced to wider range of neural circuit disorders; therefore, all basal ganglia nuclei or neural fibers can be a functional target in future and we need to prepare the basis of further development of stereotactic and functional neurosurgery. Lastly, not only macroscopic, but also a microscopic histology is prerequisite for the value of brain atlas in neuroscience research.

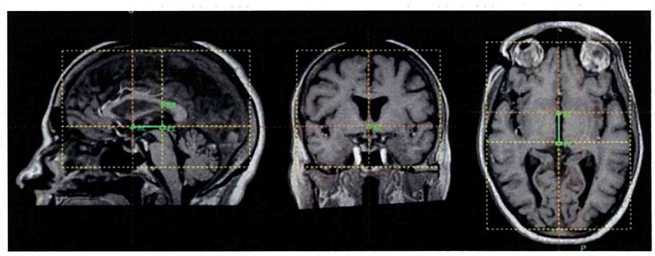

Fig. 1. A simple orthogonal magnification of classical brain atlas

Because a large and soft organ such as human brain is subject to a mechanical deformation, the production of stereotactic human brain atlases is still time-consuming and needs special instruments, environments, manpower and skills. To achieve both the 3D-consistency and high histological quality for stereotactic human brain atlases, we have established a novel method using a formalin-fixed cadaver brain of the Japanese, blade-oscillation microslicer and information engineering technique [4]. This method enabled the reconstruction of histological sections of human brain with the 3D-consistency excellent enough to make the stereotactic atlases of human brain, as well as the successful preservation of original macroscopic shape and microscopic features, such as cytoarchitecture [5]. We have extended this method in order to construct a brain simulator for safe and accurate functional neurosurgery. Neuroscience researches, such as neural circuit simulator or computational modelling, will further develop explosively when they have the organic link to the neuroanatomical knowledge database made by Brain simulator as a platform.

II. Purpose

Development of multimodal brain atlas is one of top agendas in neuroscience research [6]. The purpose of this project is to construct a brain simulator for the establishment of safe and accurate functional neurosurgery. In this sense, the following specific goals are raised in each step towards the construction of brain simulator.

1. To promote efficiency in constructing histological atlas from the whole brain from a Japanese
2. To develop non-rigid deformation of brain atlas tailored to individual patient
3. To promote data collection form MR images and the extraction of the deformation parameters
4. To register the functional data in each coordinate of the atlas
5. To simulate the functional and mechanical response to trauma, edema, surgical maneuver.

III. Plan

A. Construction of Histological Brain Atlas

We have applied a digital imaging technique and blade-oscillation microslicer to the construction of stereotactic histological brain atlas of human. Namely, the surface of a cadaver brain of a Japanese male was scanned by non-contact 3D-digitizer (VIVID910, Konica Minolta Inc., Japan) and integrated in one surface model as a digital data [4].

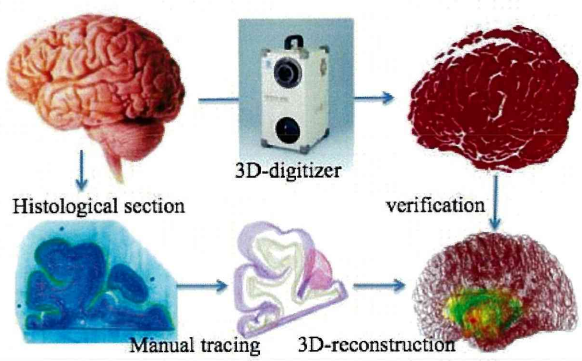

Fig. 2. A method of 3D-reconstruction of the histological sections of the Japanese male brain

The brain was divided into coronal blocks perpendicular to the intercommissural line by brain-cutting machine (VC-600, Meiko Medical Ltd., Japan) and sliced into histological sections using blade-oscillation microslicer (DTK-3000W, Dosaka-EM, Japan).

Histological sections were stained with Nissl- and Myelin-stain and they were scanned into bitmap format. After the four fiducial markers in gelatin frame were used for coregistration of section images slice to slice within a block, the surface contours, ventricles and major subcortical neural structures in the images were manually traced using 3D neuron reconstruction software, Neurolucida (MicroBrightField, Inc., USA) (Fig.2) [4],[5].

B. Construction of Computational Brain Model

Multiple T1-weighted MR brain images of normal volunteers of various ages (control) and movement disorders (dystonia or Parkinson's disease) are separately converted into brain models and the boundaries of subcortical structures visible on MR, such as white mater, ventricle, caudate and putamen, are manually traced using Neurolucida and reconstructed into one 3D model. In control and disease group (dystonia and Parkinson's disease), the regional atrophy ratios are calculated and registered to the coordinates of histological brain atlas. The regional atrophy ratio will be later used for the parameters of individual deformation of standard histological brain atlas. Furthermore, the material data and functional data reported elsewhere in the literatures will be registered to the coordinates.

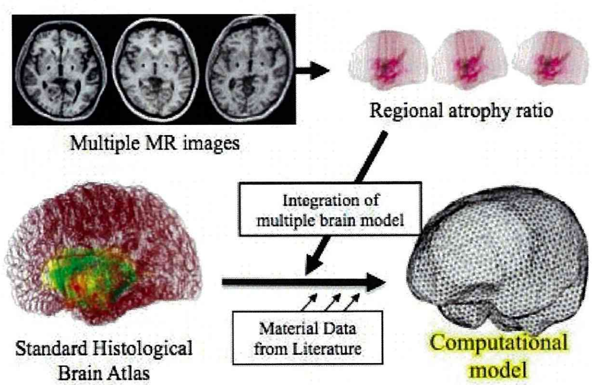

Fig. 3. A method of construction of computational brain model

C. Construction of Brain Simulator

Once a computational brain model is established, the model can be extended to work in almost all fields of neuroscience. To use a model as a simulator, the following 4 functions should be prepared; 1) tailor-made deformation to fit individual brain image, 2) anatomical platform for neural circuit model to predict a therapy effect, 3) navigation system for neurosurgery to overcome intraoperative deformation, 4) registration of functional data as an updatable storage. These functions enable us to use computational brain model directly