1 # Diffusion Tensor Tractography of Brainstem Fibers and Its

2 # Application in Pain

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21

Abstract

23   Evaluation of brainstem pathways with diffusion tensor imaging (DTI) and tractography

24   may provide insights into pathophysiologies associated with dysfunction of key brainstem

25   circuits. However, identification of these tracts has been elusive, with relatively few in vivo

26   human studies to date. In this paper we proposed an automated approach for reconstructing nine

27   brainstem fiber trajectories of pathways that might be involved in pain modulation. We first

28   performed native-space manual tractography of these fibers in a small normative cohort of

29   participants and confirmed the anatomical precision of the results using existing anatomical

30   literature. Second, region-of-interest pairs were manually defined at each extracted fiber's

31   termini and nonlinearly warped to a standard anatomical brain template to create an atlas of the

32   region-of-interest pairs. The resulting atlas was then transformed non-linearly into the native

33   space of 17 veteran patients' brains for automated brainstem tractography. Lastly, we assessed

34   the relationships between the integrity levels of the obtained fiber bundles and pain severity

35   levels. Fractional anisotropy (FA) measures derived using automated tractography reflected the

36   respective tracts' FA levels obtained via manual tractography. A significant inverse relationship

37   between FA and pain levels was detected within the automatically derived dorsal and medial

38   longitudinal fasciculi of the brainstem. This study demonstrates the utility of DTI in exploring

39   brainstem circuitries involved in pain processing. In this context, the described automated

40   approach is a viable alternative to the time-consuming manual tractography. The physiological

41   and functional relevance of the measures derived from automated tractography is evidenced by

42   their relationships with individual pain severities.

43

Introduction

44

45   The brainstem, including the midbrain, pons, and medulla, involves structures with

46   complex white matter pathways and gray matter nuclei that are concentrated in a small area.

47   Intricate brainstem circuitries and nuclei serve systems such as respiratory and cardiovascular

48   regulation, sleep and alertness, pain, posture, mood, and mnemonic functions. It is crucial to

49   understand how structural changes in small brainstem regions and circuitries may cause/alter

50   pathologies.

51   Studies of brainstem substructures using anatomical brain MRI have been complicated by

52   difficulties in detecting neuronal loss due to lack of sufficient contrast to delineate small internal

53   substructures in intensity-based images. Diffusion tensor imaging (DTI) is a noninvasive MRI

54   imaging technique that measures changes of water diffusion in white matter microstructures.

55   Fractional anisotropy (FA), one of the standard DTI indices, is known to be sensitive to detect

56   damages in orientationally organized structures (e.g. white matter fibers). Furthermore, based on

57   computing the directional information in each voxel of DTI, tractography is used to reconstruct

58   trajectories of white matter tracts that correspond to known neuroanatomy in 3-dimensional

59   space [1,2]. Diffusion tensor tractography has orientation-based contrasts and thus permits not

60   only the anatomical illustration of neural pathways, but also examines the integrity and structural

61   connectivity of a specific pathway by measuring the microstructural or fiber indices along the

62   reconstructed tracts.

63   Diffusion tensor tractography has been broadly applied in neurosurgical settings, such as

64   navigating tumor resections to avoid damage to surrounding vital neural pathways [3] and

65   providing guidance to targets of deep brain stimulation electrodes [4]. Quantitative DTI

66   measures in tractography-derived fiber bundles have also been used for detecting microstructural

67   deficits in multiple neurologic and psychiatric disorders, such as amyotrophic lateral sclerosis

68   [5], cognitive impairments and Alzheimer's Disease [6-8], and other neurologic and psychiatric

69   disorders [9]. However, tracking and isolating brainstem pathways using DTI is challenging due

70   to their spatial overlapping and crossing with other major tracts that project to cerebral cortices.

71   Previous tractography investigations pertaining to the brainstem have focused on major fiber

72   bundles between the brainstem and the cortex [10] that provided few details about specific fiber

73   tracts within the brainstem. A voxel-wise tract-based spatial statistics (TBSS) [11] approach

74   allows for comparisons of FA in "skeletons" within the brainstem. While this method can be

75   fully automated, the TBSS approach analyzes data at selected high-FA voxel locations that do

76   not necessarily belong to actual fiber tracts, and the measures at voxel level could additionally be

77   inaccurate when FA is disrupted in the presence of disease. There are, however, increasing

78   clinical needs for investigating brainstem fibers and their mnemonic functional relevance. A

79   recent study [12] has established a human brainstem fiber atlas based on tractography from a

80   large population of 488 young healthy subjects. This tractographic atlas may indeed benefit

81   clinical neurosurgical interventions, but the neuropathological imaging applications of measuring

82   FA in non-invasively isolated brainstem fibers are yet unknown. As atlas-based analyses are

83   sensitive to individual uncertainties of small fibers, tractography based on native DTI data is still

84   needed. Furthermore, the atlas was derived from data collected from healthy young adults using

85   a high angular resolution diffusion imaging (HARDI) sequence [13], which is very difficult to

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86   implement in most clinical DTI scan protocols for neurological purposes. A reliable, clinically

87   applicable, brainstem tractography and quantitative measure is thus needed.

88   The aims of this study were to 1) demonstrate the practical feasibility and

89   neuroanatomical consistency of tractography for most trackable brainstem fibers based on a

90   clinical DTI scan protocol; 2) automate the described tractographic procedure and test its

91   reliability of quantitative measures of the brainstem fibers together with manual tractography;

92   and 3) use automated tractography to assess the association between the integrity of brainstem

93   tracts and chronic pain in an attempt to identify brainstem pathways that are specifically involved

94   in pain regulation and processing.

95

Materials and Methods

Participants and Pain Evaluation

98   Seventeen participants, age between 39 and 59 years (mean age = 49.7 ± 5.2, 16 males),

99   without brainstem lesions on MRI, were selected from patients who were recruited at the

100   California War Related Illness and Injury Study Center (WRIISC-CA) at the Veterans Affairs

101   Palo Alto Health Center (VAPAHCS) between March and October of 2017. All participants

102   were combat veterans, 9 of whom were deployed to the 1991 Persian Gulf War, 6 to Afghanistan

103   and/or Iraq as part of Operations Iraqi Freedom and Enduring Freedom, and 2 to other combat

104   zones. All participants had endorsed diffuse combat-related symptoms via self-reported

105   questionnaires that meet the criteria for chronic multi-symptom illness (CMI) [14]. Twelve

106 veterans, within 24 hours before their MRI scans, had filled out item 6 from the Brief Pain

107 Inventory Short Form (BPI) [15], which involved self-report of their current pain level ('pain

108 right now'). All veterans also had filled out self-reported questionnaire of the worst pain they can

109 recall in a month prior to MRI ('worst pain in last month'). Both pain scores were leveled from

110 scale from 0 (none) to 10 (extreme), as an overall pain level regardless of locations. All aspects

111 of the study were approved by the Stanford University and VAPAHCS Institutional Review

112 Boards, and written informed consent to analyze clinical data was obtained from all participants.

Structural MRI and DTI acquisition

114   Brain imaging data were collected at the Veterans Affairs Hospital in Palo Alto, CA,

115 USA, using a 3 tesla GE Discovery MR750 scanner with an 8 channel GE head coil. High-

116 resolution T1-weighted images (T1WI) were acquired using three-dimensional spoiled-gradient

117 recalled acquisitions (3D-SPGR) in steady state (136 sagittal slices, TR/TE = 7.3/3.0 ms; flip

118 angle = 11°; field of view = 250 mm; slice thickness = 1.2 mm with 0.6 mm slice gap; acquisition

119 matrix = 256 × 256; number of excitations = 1.0; resolution = 1.05 mm × 1.05 mm × 0.60 mm).

120 T2-weighted images (T2WI) were acquired using fast spin-echo (FSE) sequences with TR/TE =

121 7652/98.4 ms, with 0.45 x 0.45 mm^2 in-plane resolution and 3.5 mm slice thickness, for 47 axial

122 slices. The T2WI images were collected for correcting geometric distortions of DTI relative to

123 the structural T1WI. Diffusion tensor imaging scans were acquired with a 2D single-shot EPI

124 sequence with TR/TE = 6600/84.1 ms, 1 x 1 x 2.5 mm^3 resolutions, with 59 contiguous axial

125 slices to enable full brain coverage. Ten scans without diffusion gradients (b0) and scans along

126 60 sensitization directions using diffusion-weighting gradients (DWI) with b-value of

127 1000s/mm^2 were acquired for DTI reconstruction.

DTI Processing Pipeline

129   The T1WI and DTI scans were initially checked for visual artifacts, and subsequently

130   processed using an in-lab image processing pipeline, shown in Fig 1. After quality control,

131   structural (T1WI/T2WI) and DTI data sets collected from 300 WRIISC-CA participants, who

132   were veterans aged between 30 and 80 years, were processed through this pipeline to create

133   customized templates and atlases in Montreal Neurological Institute (MNI) space. Creating

134   customized templates using averaged images from the same study cohort (e.g. WRIISC-CA) is

135   recommended [16,17], because the minimal cross-subjects variation results largely improve the

136   registration accuracy. This in-lab pipeline included the following: 1) correction for head motion,

137   susceptibility artifacts, and eddy-current distortions in native space using scripts from the

138   Stanford Vision Imaging Science and Technology Lab (VISTA Lab;

139   https://vistalab.stanford.edu/software/). Maps of DTI metrics, such as fractional anisotropy (FA),

140   mean diffusivity, radial diffusivity, axial diffusivity, were then computed using FDT software in

141   FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT). 2) Geometric distortions of DTI relative to

142   anatomical MRI (T1WI and T2WI) in subject's native space, which are particularly apparent in

143   the brainstem (see supplementary S1 Fig), were corrected using Advanced Normalization Tools

144   (ANTs) [18], through the following procedure: The initial b0 image was aligned to same

145   subject's T2WI using a smooth deformation algorithm and both images were subsequently

146   transformed to anatomical T1WI using affine transformation. After distortion correction, the

147   non-linear distortions between DTI frame and anatomical T1WI were effectively reduced. Thus,

148   the accuracy of anatomical landmark assignment within individual spaces was improved

149   (Supplementary S1 Fig). 3) Using nonlinear deformation algorithms in ANTs, all participants'

150   T1WI were diffeomorphically warped to the MNI space and averaged to create customized T1

151   and DTI templates. Then, an atlas with anatomical parcellations of whole-brain gray matter,

152   white matter regions defined according to the JHU-DTI-MNI (Type I WMPM) [19] was spatially

153   warped onto the customized template to provide anatomical labels for aiding the definition of

154   regions-of-interest (ROIs) onto the templates. 4) The inverse of the above transformations was

155   then used to transform anatomical parcellations and regions-of-interest (ROIs) specified in the

156   MNI space to subjects' native DTI space.

157

158   **Fig 1**. **DTI processing and brainstem tractography flowchart.** In-lab image processing

159   pipeline for registration and atlas building based on structural MRI and DTI from 300 WRIISC-

160   CA participants.

161

Manual Tractography of The Brainstem Fibers

163   Manual brainstem tractography was performed on original b0 and DWI data using

164   Diffusion Tensor Toolkits (DTK) (http://dti-tk.sourceforge.net/pmwiki/pmwiki.php) and

165   TrackVis software (http://www.trackvis.org/), with a default linear least-squares fitting method

166   in DTK. As a default function, DTK/TrackVis computed mean FA and fiber density (i.e. the

167   streamline numbers) in each voxel of a fiber tract that resulted from tractography as the outcome

168   measures. Manual tractography was performed using TrackVis. Each brainstem tract was

169   initiated by placing a 'seed' ROI in the brainstem, and a 'target' ROI in the distant end.

170   Deterministic tractography using a fixed step-length streamline propagation [20] algorithm was

171   performed with an 'AND' option to reconstruct trajectories between both ROIs, with the

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172   minimum length and curvature thresholds of 10 mm and 30º, respectively. Seeds and targets

173   were manually drawn by an experienced radiologist in accordance to known brainstem anatomy.

174   The initial tractography consistently resulted in the intended trajectory, but additionally also

175   resulted in other trajectories, some of which potentially being artifacts, and some that could be

176   other neighboring pathways. In these cases, manual adjustments of 'NOT' ROIs were used to

177   eliminate them. On the other hand, if the initial tractography had no trajectory results because the

178   'seed' and 'target' ROIs were too far apart, the distance between the ROI pairs was adjusted

179   within their anatomical landmarks until any trajectories consistent with known anatomy could be

180   achieved. Because manual tractography is extremely time-consuming, DTI data of 7 participants

181   randomly selected from all participants were processed with manual tractography. Fig 2 depicts

182   the ROI placements and reconstructed streamlines of nine fiber tracts that travel through the

183   brainstem. For comparison with sectional anatomy, cross-sectional slices at the lower and upper

184   brainstem panel of the color-oriented anisotropic map, six representative brainstem fibers, and

185   corresponding neuroanatomy are shown in Fig 3. Overall, nine pairs of brainstem fiber tracts

186   were tracked:

187

188   **Fig 2. Brainstem fiber tracts and manual ROI placement.** Placement of ROI-pairs ("Seed"

189   and "target") and their fiber outputs performed on TrackVis. Abbreviations: MLF= medial

190   longitudinal fasciculus; DLF = dorsal longitudinal fasciculus; SCP = superior cerebellar

191   peduncle; MFT = medial forebrain tract; NST = nigrostriatal tract; FPT = frontopontine tract;

192   CST= corticospinal tract; STT = spinothalamic tract; POTPT = parieto-, occipito-, and

193   temporopontine Tract.

9

194 **Fig 3. Representative brainstem fiber tracts and corresponding neuroanatomy in two**

195 **brainstem levels.** *Left*: color-coded anisotropic map (red: left-right oriented; blue: superior-

196 inferior oriented; green: anterior-posterior oriented); *Middle*: six brainstem reticular tracts

197 superimposed on a T1WI; *Right*: corresponding anatomic sections. Abbreviations: CP = cerebral

198 peduncle; ML= medial lemniscus; STT = spinothalamic tract; MCP = middle cerebellar

199 peduncle; SCP = superior cerebellar peduncle; MLF= medial longitudinal fasciculus); DLF =

200 dorsal longitudinal fasciculus; MFT = Medial Forebrain Tract; NST = nigrostriatal tract; LC

201 =locus coeruleus; PAG = periaqueductal gray matter. The brainstem anatomical pictures are

202 downloaded from the web site

203 (http://www.dartmouth.edu/~rswenson/Atlas/BrainStem/index.html) with permission of the

204 author.

205

206   1) Medial longitudinal fasciculus (MLF; Fig 2A. – yellow): the seed ROI was placed in

207 the area anterior to the 4th ventricle floor, including the inferior cerebellar peduncle (ICP) and

208 the medial lemniscus (ML). The target ROI was placed in the midbrain (MidB), encompassing

209 the ventral tegmentum area (VTA) and the red nuclei (RedN). In the brainstem, the reconstructed

210 fibers travelled ventrally to the aqueduct and the 4th ventricle, slightly laterally to the midline,

211 where the Raphe nuclei are located (Fig 3). This pattern was consistent with previous

212 tractography of healthy human brainstems and known anatomy of MLF [12,21].

213   2) Dorsal Longitudinal Fasciculus (DLF; Fig 2A – red): the seed ROI was placed in the

214 area anterior to the aqueduct, including the periaqueductal gray matter (PAG) and surrounding

215 area as well as the locus coeruleus (LC). The target ROI was placed in the dorsal MidB,

10

216   including the mammillothalamic tract, which is located laterally to the cerebral aqueduct. The

217   reconstructed fibers connected between the LC and the hypothalamus, travelled ventrolaterally to

218   the cerebral aqueduct, and ran dorsocaudally to the MLF (Fig 3). The tractography pattern of

219   DLF was consistent with known anatomy and a previously-described tractography atlas [12,21].

220   3) Superior Cerebellar Peduncle (SCP; Fig 2A – cyan): The seed ROI was placed in the

221   area posterior to the 4th ventricle floor and aqueduct, including white matter in the superior

222   cerebellar peduncle and the cerebellar dentate. The target ROI was placed in the ventral

223   thalamus. The reconstructed fibers connected the dentate nuclei and the thalamus, including non-

224   decussated and decussated dentato-rubrothalamic tracts. This tract and its anatomy has been

225   reported in many previous tractography studies of the human brain [12, 22].

226   4) Nigrostriatal tract (NST; Fig 2B – pink): The seed ROI was placed in the MidB,

227   including the entire substantia nigra (SN). The target ROI was placed in the inferior level of the

228   putamen and the globus pallidus formation (Put/GP). This small fiber travelled from the SN

229   dorsomedially to the subthalamic nuclei (STN) and turned dorsolaterally toward the ventral

230   Put/GP. This pattern was consistent with previous tractography findings and known anatomy of

231   the NST [23].

232   5) Medial Forebrain Tract (MFT; Fig 2B – green): The seed ROI was placed in the

233   ventral MidB area anterior to the aqueduct, including ventral tegmental area (VTA). The target

234   ROI was placed in the inferior frontal gray and white matter area, including the rectus,

235   orbitofrontal, and the prefrontal cortices. This tract originated in the VTA, extended

236   dorsolaterally toward the nucleus accumbens (NAc) and anterior thalamic radiation (ATR,

237   located in the anterior limb of the internal capsule), and finally projected to the orbitofrontal

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238 cortex (OFC) and the dorsolateral prefrontal cortex (DLPFC). The tractography and

239 neuroanatomy of MFT has been demonstrated in previous papers [24,25].

240   6) Frontopontine Tract (FPT; Fig 2C – light pink): The seed ROI was placed in the

241 ventral medial 1/3 of the cerebral peduncle (CP). The target ROI was placed in the middle and

242 superior frontal gray and white matter, including the supplementary motor and the premotor

243 areas, but excluding the primary motor area. The FPTs, originate from the frontal lobe, descend

244 through the anterior limb of the internal capsule, and end in the pontine nuclei, have been

245 described in many neuroanatomy textbooks and tractography studies [26].

246   7) Corticospinal Tract (CST; Fig 2C – blue): The seed ROI was placed in the middle 1/3

247 of the CP. The target' ROI was placed in the precentral gray and white matter, which

248 corresponds to the primary motor area. The CST is the primary motor pathway descending from

249 the motor cortex innervating muscles controlling skilled voluntary movement. This ROI

250 placement of tracking the CST is in line with previously well-described strategies to reconstruct

251 CST using DTI tractography [26].

252   8) Spinothalamic Tract (STT; Fig 2C – orange): The seed ROI was placed in the ML and

253 the surrounding area. The target ROI was placed in postcentral (sensory) gray and white matter,

254 corresponding to the primary somatosensory area. The STT rose up the brainstem ML area,

255 projected to thalamus, and eventually reached the sensory cortex. The tractography of this

256 pathway has been demonstrated previously [12].

257   9) Parieto-, Occipito-, and Temporopontine Tract (POTPT; Fig 2C – purple): The seed

258 ROI was placed in the laterocaudal third of the CP. The target ROI was placed broadly in the

259 parietal, occipital, and temporal gray and white matter parcellations. The parietopontine and

260 occipitopontine tracts passed the posterior limb of the internal capsule, retrolenticular part of the

261 internal capsule (RLIC), and the dorsal thalamus; the temporopontine tract travelled medially to

262 the hippocampoamygdala formation. These patterns were in agreement with a previous report

263 [12].

264   The tractographic anatomy, possibly related neurotransmitter systems and functions of

265 the nine tracts reconstructed from diffusion tensor tractography are summarized in Table 1.

266

267 **Table 1**. **Anatomy, possible associated neurotransmitters and functions of brainstem**

268 **circuits.**

Brainstem tracts	Gray/white matter structures in the brainstem tractographic pathways	Possible Neuro-transmitter	Related Functions
MLF	Begin: ICP and ML; Pass: lower raphe nuclei, ML; End: MidB and RN	Serotonin, Noradrenaline	wakefulness, pain control, maintaining posture, cardiovascular control, eye movement
DLF	Begin: PAG and Surrounding area; Pass: LC, PAG; End: hypothalamus; mamillary body	Serotonin, Noradrenaline, Histamine	wakefulness, attention, consciousness, stress and reward, pain
SCP	Begin: SCP and cerebellar dentate nuclei; Pass: PPN; End: RN and thalamus	Acetylcholine, GABA	sleep, cognition, mood, attention, arousal, voluntary limb movements, locomotion

NST	Begin: SN Pass: VTA, STN End: putamen and GP	Dopamine, Serotonin	motor function, mood, pleasure and reinforcement
MFT	Begin: MidB and VTA Pass: NAc, ATR End: rectal, orbitofrontal, and prefrontal cortices	Acetylcholine	mood, word, reward and pleasure
FPT	Begin: medial 1/3 of the CP Pass: ALIC, SCR, frontal cortices End: superior frontal, supplementary motor area, and the premotor areas	Non-reticular system	control of movement, facial movement
CST	Begin: middle 1/3 of the CP Pass: PLIC End: precentral (primary motor) cortex	Non-reticular system	movement of body muscles
STT	Begin: ML Pass: dorsal spinal cord, ML, thalamus End: postcentral (sensory) cortex	Non-reticular system	sensory of temperature, pain
POTPT	Begin: lateral 1/3 of the CP Pass: ventral medulla, RLIC, dorsal thalamus, amygdala and hippocampus End: parietal, occipital, and temporal cortex	Non-reticular system	variety of functions: working memory, learning, visual, auditory

269 Abbreviations: ICP = inferior cerebellar peduncle; ML = medial lemniscus; MidB = midbrain;

270 RN = red nucleus; PAG = periaquaductal grey matter; LC = locus coeruleus; SCP = superior

271 cerebellar peduncle; PPN = pedunculopontine nucleus; SN = substantia nigra; VTA = ventral

272 tegmental area; STN = subthalamic nucleus; GP = Globus pallidus; NAc = Nucleus Accumbens;

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273   ATR = anterior thalamic radiation; CP = cerebral peduncle; ALIC = anterior limb and genu of the

274   internal capsule; SCR = superior coronal radiata; PLIC = posterior limb of the internal capsule; RLIC =

275   retrolenticular part of the internal capsule.

276

Automated Brainstem Tractography

278   A common practice of automated-tractography is to apply pre-defined ROI-pairs from the

279   MNI atlas space onto individual DTI data to extract only those streamlines that run through both

280   ROI ends. However, most of the brainstem fiber tracts (e.g. MLF, DLF, NST and MFT) do not

281   have a previously established ROI atlas or parcellations in MNI space. In this study, we use the

282   manually-defined ROI-pairs and fiber results to build the pre-defined ROI templates of the

283   brainstem tracts and subsequently tested the reproducibility of these tractography results in

284   comparison with manual tractography. Briefly, the procedure of building the templates of ROI-

285   pairs in MNI space included: 1) employing the in-lab imaging processing pipeline to transfer the

286   manually defined ROI pairs and the 9-brainstem fibers into MNI space. 2) in MNI space, the

287   normalized ROI pairs of each fiber were averaged and binarized to create the pre-defined ROI-

288   pair templates; the normalized fibers were also averaged to create fiber templates as the

289   anatomical references; 3) to further improve anatomical precision of each fiber isolation,

290   minimal adjustments of the ROI templates were performed including: (i) dilating the ROI

291   boundaries to ensure the ROI-pairs fully cover the termini of each fiber template, (ii) eroding

292   ROI boundaries between two adjacent ROIs (e.g. the boundaries of MLF and DLF) to eliminate

293   overlapping tractographic results; (ii) limiting the dilation and erosion of the ROI templates

294   within the anatomical landmarks based on the JHU-DTI-MNI atlas (also described in Table 1 as

15

295   the 'begin' and 'end' regions). Once the templates of ROI-pairs were built in MNI space, they

296   were transferred backwards through the inverse transformation of the in-lab image processing

297   pipeline onto all participants' native DTI space to launch individual brainstem trajectories using

298   TrackVis between the assigned ROI-pairs. The overall processing time of this automated

299   tractography procedure for tracking the 9 brainstem fiber pairs was approximately 30~40

300   minutes per subject.

Test-retest of the Quality and Quantity for the Brainstem Tracts

302   There is currently no comparative standard for the assessment of anatomical precision of

303   tractographic approaches. Defining false positive or negative fiber tracts from tractographic

304   results relies on prior anatomical knowledge. Therefore, tractography with ROIs that have been

305   manually defined by experts using such anatomical knowledge and extensive training, although

306   imperfect, is a conventionally accepted validation method.

307   While the presence of unexpected fiber lines (i.e. false positive fibers) can be removed by

308   the tractographer based on prior anatomical knowledge during tractography, the absence of

309   expected fiber lines (i.e. false negative fibers) is still a problem that can result in failure of

310   quantitative measurements along existing fiber lines. In this study, we tested the 'success rate'

311   for each brainstem fiber in terms of the subjects' numbers that successfully presented a certain

312   brainstem fiber, divided by the subjects' numbers that brainstem tractography was performed

313   based on either manual or automated tractography. The 'success rate' reflects the feasibility of

314   brainstem tractography in its utilization for quantitative measurement.

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315   Next, two quantitative scalar indices were obtained in each presented brainstem tract as

316   the major outcome measures: (1) The FA index, which indicates the microstructural integrity of

317   fiber connection, based on the mean FA per 1 mm^3 voxel in each brainstem tract. The fiber

318   density index, which represents the weights of reconstructed fibers, based on the mean

319   streamline numbers per 1 mm^3 voxel in each brainstem tract [27]. We used an intra-class

320   correlation coefficient (ICC) to test if quantitative measures based on automated tractography

321   could reliably reproduce the same measure based on manual tractography. The reproducibility

322   explains how reliable certain outcome measures based on automated tractography could be

323   applied as an alternative approach to manual tractography.

324

325   Results

326   Success Rate and Reproducibility of Quantitative Tractography

327   Supplementary Table S1 lists the success rates of manual and automated tractographic

328   performances in each brainstem tract. For manual tractography, most of the brainstem fibers

329   were successfully detected. Some failed tracking occurred in fibers that travelled through long

330   distances or areas susceptible to artifacts, crossing-fibers and other quality problems. Automated

331   tractography achieved a slightly smaller, but similar success rate as the manual tractography.

332   Both approaches had 85-100% success rates, which allow for the outcome measures and analyses

333   based on our conventional DTI quality.

334   Supplementary Table S1 also summarizes reproducibility between manual and automated

335   tractographic approaches. The FA in fibers measured by automated tractography reliably

336   reproduced those measured by manual tractography. On the other hand, fiber density from

337   automated tractography could marginally reproduce the results from manual tractography.

Relations between DTI and Pain levels

339   Linear regression models were used to examine the relationships of FA or fiber density in

340   each brainstem tract with the two self-reported pain levels (i.e. 'pain right now' and 'worst pain

341   last month'), separately. Table 2 and Fig 4 show that, significant associations were observed

342   between both pain levels and FA in the DLF and MLF, but not in other brainstem tracts.

343   Specifically, after controlling for age and gender, per unit increase in 'pain right now' level

344   corresponded to a 3.48% (CI_{95}: 0.5 to 6.4%, $p = 0.026$) FA reduction in DLF and a 3.02% (CI_{95}:

345   0.7 to 5.4%, $p = 0.017$) FA reduction in MLF. Furthermore, per unit increase in 'worst pain last

346   month' corresponded to a 2.30% (CI_{95}: 0.7 to 3.9%, $p = 0.008$) FA reduction in DLF and 1.87%

347   (CI_{95}: 0.4 to 3.4%, $p = 0.019$) FA reduction in MLF. No significant relation between pain levels

348   and fiber density was detected.

349   **Table 2. Linear regressions between FA of brainstem fibers and pain levels.**

Tract name	Pain Right Now (n=12)			Worst Pain Last Month (n=17)		
	Mean (S.E.) % FA change rate /scale increase	Effect-size	*p*-value	Mean (S.E.) % FA change rate /scale increase	Effect-size	*p*-value

MLF	**-3.02 (1.04)**	**-1.75**	**0.017**	**-1.87 (0.71)**	**-1.32**	**0.019**
DLF	**-3.48 (1.30)**	**-1.61**	**0.026**	**-2.30 (0.75)**	**-1.53**	**0.008**
SCP	-7.32 (3.28)	-1.34	0.053	-2.98 (1.47)	-1.02	0.062
NST	-0.72 (1.50)	-0.15	0.644	-2.47 (1.83)	-0.75	0.204
MFT	0.94 (0.62)	1.07	0.180	0.30 (0.53)	0.33	0.582
FPT	-0.60 (0.82)	-0.47	0.482	0.35 (0.63)	0.29	0.588
CST	0.21 (1.05)	0.12	0.860	0.84 (0.95)	0.51	0.393
STT	-0.59 (0.66)	-0.53	0.400	0.01 (0.68)	0.01	0.989
POTPT	-0.79 (1.39)	-0.38	0.586	-0.04 (1.12)	-0.02	0.970

350   Mean (S.E.) FA change rate (%) associated with per increased pain scale, effect size and

351   significance that estimated based on linear regression test for each brainstem fiber.

352   Effect size = (2 * T-value) / SQRT (degree of freedom)

353

354   **Fig 4**. **Relations between FA and Pain levels.** Scatter plots of relations between FA of the MLF

355   and DLF tracts and two pain scales.

356

Discussion

358   In this study, we introduced an automated tractographic approach based on a predefined

359   ROI atlas for efficiently tracking and isolating small white matter tracts in the human brainstem.

360   Our results demonstrated the usefulness of quantitative tractography in analyzing the relationship

361   between brainstem tracts and chronic pain. This application was used to detect several brainstem

362   fibers and diminished WM integrity levels were associated with pain severity.

19

Brainstem Tractographic Anatomy and Neurological Implications

363

364   Most brainstem fibers could be subdivided into three types of circuits: 1) the

365   corticopontine fibers that travel between spinal cord, pons, and the neocortices, including FPT,

366   CST, STT, POTPT (Table 1 and Fig 2C); 2) the reticular tracts that interconnect nuclei that are

367   located throughout the brainstem and the midbrain, including DLF, MLF, SCP, MFT and NST

368   (Table 1 and Fig 2A and 2B); and 3) the pontinopeduncle tracts that run transversely between

369   pons and cerebellar peduncles including the middle cerebellar peduncle, the inferior cerebellar

370   peduncle and pontine crossing tracts. Among this traffic, the reticular tracts have major clinical

371   significance in indicating essential neurotransmitter functions (Table 1 and Fig 5). Manual and

372   automatic tractography of the corticopontine fibers and pontinopeduncle tracts have been well-

373   established previously. By contrast, tractography of the reticular tracts needs to be further

374   established as these fibers are thought to contain a variety of autonomic functions through

375   corresponding neurotransmitter pathways.

376

377   **Fig 5. Illustration of brainstem reticular tracts.** Conceptual sketch according to the DTI

378   tractography of the brainstem reticular tracts, their anatomy, fiber-linked gray matter centers and

379   potentially associated functions.

380

381   MLF is generally known to connect the cranial nerves (III, IV, and VI) which are

382   involved in eye movement, as well as the stabilization of the head and neck. MFL is also known

383   to send parasagittal fibers from the dorsal raphe nuclei, which serve as a center of serotonergic

20

384   neurons [28]. The role of MLF in modulating some autonomic, reproductive and excretory

385   functions, as well as modulating perception to pain needs to be investigated. The DLF that links

386   the hypothalamus, periaqueductal gray matter, locus coeruleus, and the tractus solitarius in the

387   medulla, carries both ascending and descending fibers. This tract is considered to be a pathway

388   regulating the sleep–wake cycle, mood, and cognition [29,30]. DLF is also related to pain control

389   [31]. SCP is a large bundle of projection fibers arising chiefly from the cerebellar dentate nuclei,

390   ascending along the posterior floor of the forth ventricle, and ending in the red nucleus and

391   thalamus. SCP plays a major role in the coordination of movement of the ipsilateral limbs.

392   Previous studies [32,33] also suggest that SCP contains GABAergic fibers. The NST is a

393   dopaminergic pathway that is involved in Parkinson's disease. Individual tractography-guided

394   quantitative DTI measures of this tract have been suggested as a promising marker for

395   identifying Parkinson's disease and such measurements may correlate with the severity of motor

396   dysfunction [34-36]. NST is also suggested to control depressive fatigue states in Parkinson's

397   Disease [37,38]. MFT, which connects the brainstem to nucleus accumbens, anterior thalamic

398   radiation, and dorsal and medial prefrontal cortices, is involved in mood, the reward system, the

399   appetitive motivational seeking system and euphoria [39]. The relation of MFT and major

400   depressive disorder has been well-documented through DTI [24], and MFT tractography is also

401   used for targeting deep brain stimulation in depression [25].

402   Given the variety of the reticular fibers and their corresponding functions, brainstem DTI

403   tractography may be highly informative in the diagnosis and study of a range of neurological

404   conditions. Our newly developed brainstem tractographic approach allows the correlation

405   analyses for clinical measures and quantitative DTI measures of fiber tracts known to be

406   involved in those neurological dysfunctions.

Quantitative Brainstem Tractography and Pain

Much of the DTI and FA studies have been focusing on examining differences in white matter microstructure in chronic pain vs. non-pain controls, and correlating FA with pain levels (see a recent review paper [40]). Based on TBSS, pain-related FA reductions have been detected in multiple white matter tracts along diffuse 'white matter skeletons', including the internal and external/extreme capsules, corpus callosum, cingulum, thalamic radiation, and brainstem white matter, primary somatosensory and motor cortices, orbitofrontal cortex [41,42], rostral anterior cingulate, and dorsal lateral prefrontal cortex [43]. TBSS and most voxel-based analytic approaches, however, are known to be highly sensitive to registration errors and may produce false positive findings [44]. Moreover, alterations in most cerebral white matter regions could also be observed in comorbid neurological symptoms such as cognitive or mood impairments, but are not specifically responsible for pain [45]. In this context, it is essential to identify key white matter circuits involved in pain perception and regulation, and to explore the potential disruptions of these key pathways in relation with pain severity levels. Electrophysiological [46] and functional MRI studies [47] have shown that stimulus-evoked pain is associated with activity in several brain areas ('pain centers') and pathways linking these areas. However, these observations did not address the actual tracts and their structural connections in association with pain states. According to brainstem anatomy, both the 'ascending (bottom-up) pathway' – which sends the nociceptive flow from the spinal cord to cerebral cortices, and the 'descending (top-down) pathway' – that supports a top-down role in modulating the nociceptive signals, are involved in the brainstem fiber traffic [48]. A previous DTI study [49] demonstrated that fiber tracts originating in the periaquaductal grey (PAG) that project towards other brain areas, which may represent the descending pain control pathway, can be successfully visualized using

22

430   probabilistic tractography. however, this study did not provide specific fiber isolation and

431   quantitative measures such as FA in subjects with pain. Our study further isolated this theoretical

432   'descending pathway' to mainly overlap with the DLF and MLF tracts. We found that

433   diminished FA in these tracts is associated with both ongoing pain (i.e. 'pain right now') and

434   chronic pain (i.e. 'pain in last month') levels. These findings, suggest that quantitative brainstem

435   tractography is a promising marker for investigating pain regulation mechanisms. Further

436   analyses based on larger patient populations and healthy controls are needed to validate these

437   preliminary findings.

Advantages of Automated Brainstem Tractography

439   This newly developed automated brainstem tractography has several merits: First, this

440   approach has been shown feasible to be applied, implemented and performed on conventional

441   DTI scans. Tracking the brainstem functional fibers on DTI is known to be difficult, and the

442   detection of those small fiber anatomies depends on to the quality of acquisition including

443   resolution, directions, controlling of noise and crossing-fibers. Existing approaches that provide

444   comprehensive brainstem tractography have been established based on sophisticated diffusion

445   acquisitions such as HARDI, diffusion spectrum (DSI) [50], etc., which provide limited clinical

446   utility. In this study, we applied brainstem tractography using a readily available and sufficiently

447   short (8 min) conventional DTI sequence, and demonstrated efficient success rates based on

448   either manual or automated tractographic approaches. This suggest that the automated

449   tractography could be adopted for quantitative measures in most clinical DTI protocols that use

450   conventional resolutions (4~8 mm^3 voxel size) and directions (30~80). Second, this automated

451   tractographic approach provides reproducible measures that overcome known difficulties

452   associated with manual tractography. Manual brainstem tractography is time consuming,

453   depends on individual experience, and may suffer from reproducibility problems. Most existing

454   automated tractography programs [10,51-53] have been used for representing tract trajectories of

455   several major cerebral pathways with known anatomy. Automated brainstem tractography is

456   currently not available due to a lack of atlas-segmented ROIs of brainstem small regions. Our

457   study developed this automated tractographic approach based on pre-defining ROI pairs in the

458   standard MNI space, with a combination of a high-quality DTI and MRI image registration

459   pipeline. We demonstrated that this approach achieves similar FA measures as manual

460   tractography while offering significant benefits through elimination of subjective rater bias,

461   greatly reduced processing times, and the feasibility for quantitative analyses of large numbers of

462   DTI data.

Limitations

464   The work presented in this paper has several limitations: first, we did not identify all

465   brainstem tracts: 1) Tractographic visibility is subject to the quality of DTI scans. Small tracts,

466   such as the rubrospinal and the central tegmental tracts, previously addressed by Meola and

467   colleagues [12], are not identifiable due to the limited quality of the DTI scan protocol used

468   herein. 2) Other pain-specific fibers connecting the PAG to cerebral cortices, described by

469   Hadjipavlou and colleagues [49], were not included in our approach, because the anatomical

470   certainty and practical feasibility of these fibers need to be validated yet. 3) We did not include

471   the pontinocerebellar, pontinomedulla, pontinospinal tracts, or cranial nerves into this approach

472   due to the coverage of the brain scans and consideration of the susceptibility artifacts near the

473   skull-base that may confound local fiber tracking. 4) We also did not perform tractography of the

474   middle or the inferior cerebellar peduncles, as well as the pontine crossing tracts because of a

475   lack of knowledge regarding the physiological and functional relevance of these transverse

476   fibers. Another limitation is that we did not test other microstructural measures (e.g. mean,

477   radial, axial diffusivities) in the brainstem fibers, although these measures might provide

478   complementary information about fiber integrities. Nevertheless, quantitative DTI changes of

479   specific brainstem fibers provide a promising biomarker in chronic pain. Further validation is

480   warranted using this automated tractographic approach in larger populations, including more

481   detailed pain evaluations and a more comprehensive consideration of all available DTI metrics.

482

Conclusions

484   This study developed an automated approach for tracking brainstem fibers based on DTI.

485   This approach is feasible for quantitative analyses and is an alternative to the very time

486   consuming manual tractography. We demonstrated the utility of this approach in correlating the

487   disruptions of some key brainstem tracts with individual pain severities. Further applications of

488   this brainstem tractographic approach may aid in the exploration of clinical implications of these

489   fibers in association with chronic pain syndromes, sleep disturbances, mood alterations,

490   autonomic disturbances and other neurological disorders.

491

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495

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499

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Supporting Information

660   **S1 Fig**. **Example of distortion corrections through image processing pipeline.** Intrinsic

661   distortion correction between individual FA map (green color) and structural T1WI (gray color)

662   improves registration accuracy in brainstem area (arrowed and zoomed areas).

663   **S1 Table. Results of test-retest for the brainstem tractographic approaches.** The success

664   rates of each manual and automated tractographic performance, and the reproducibility of each

665   outcome measure (FA and fiber density) between manual and automated tractography.
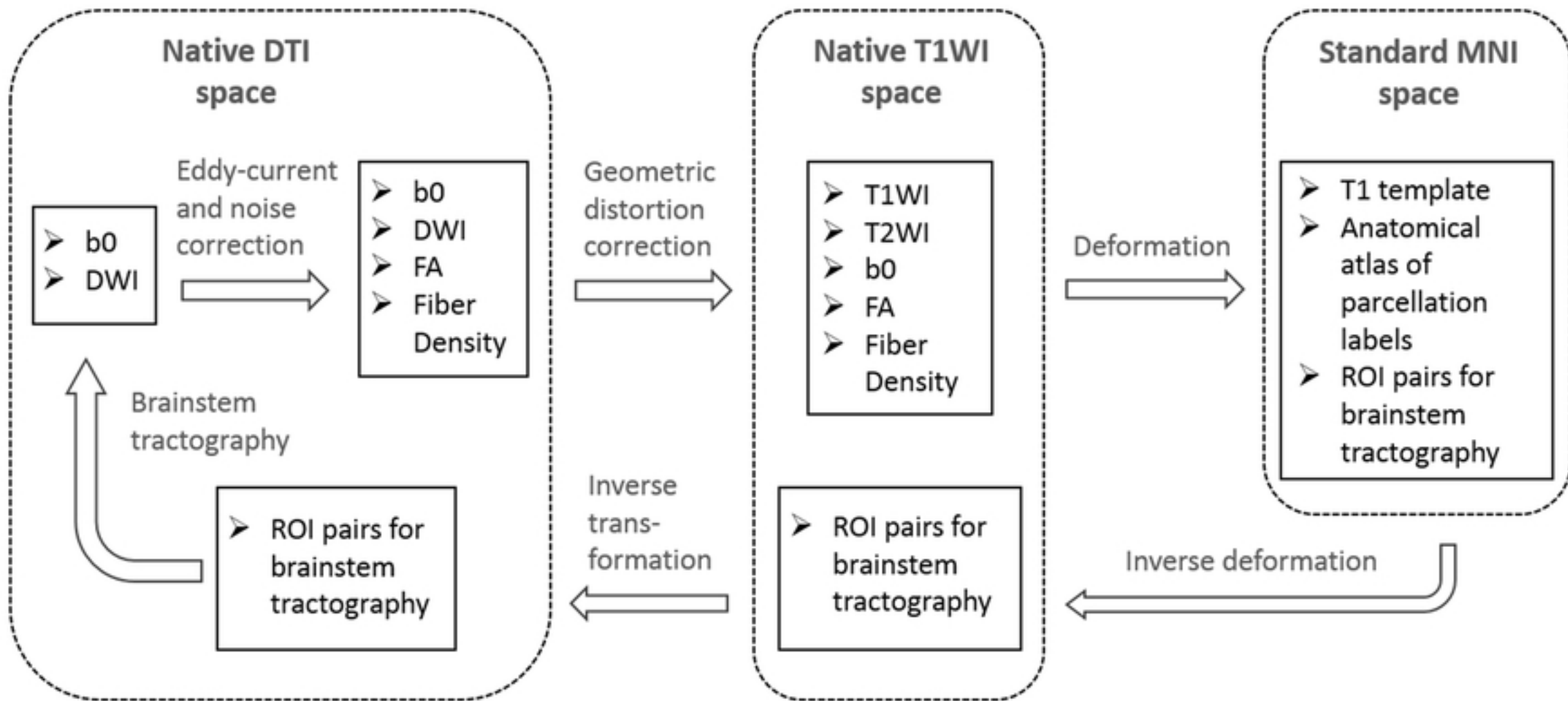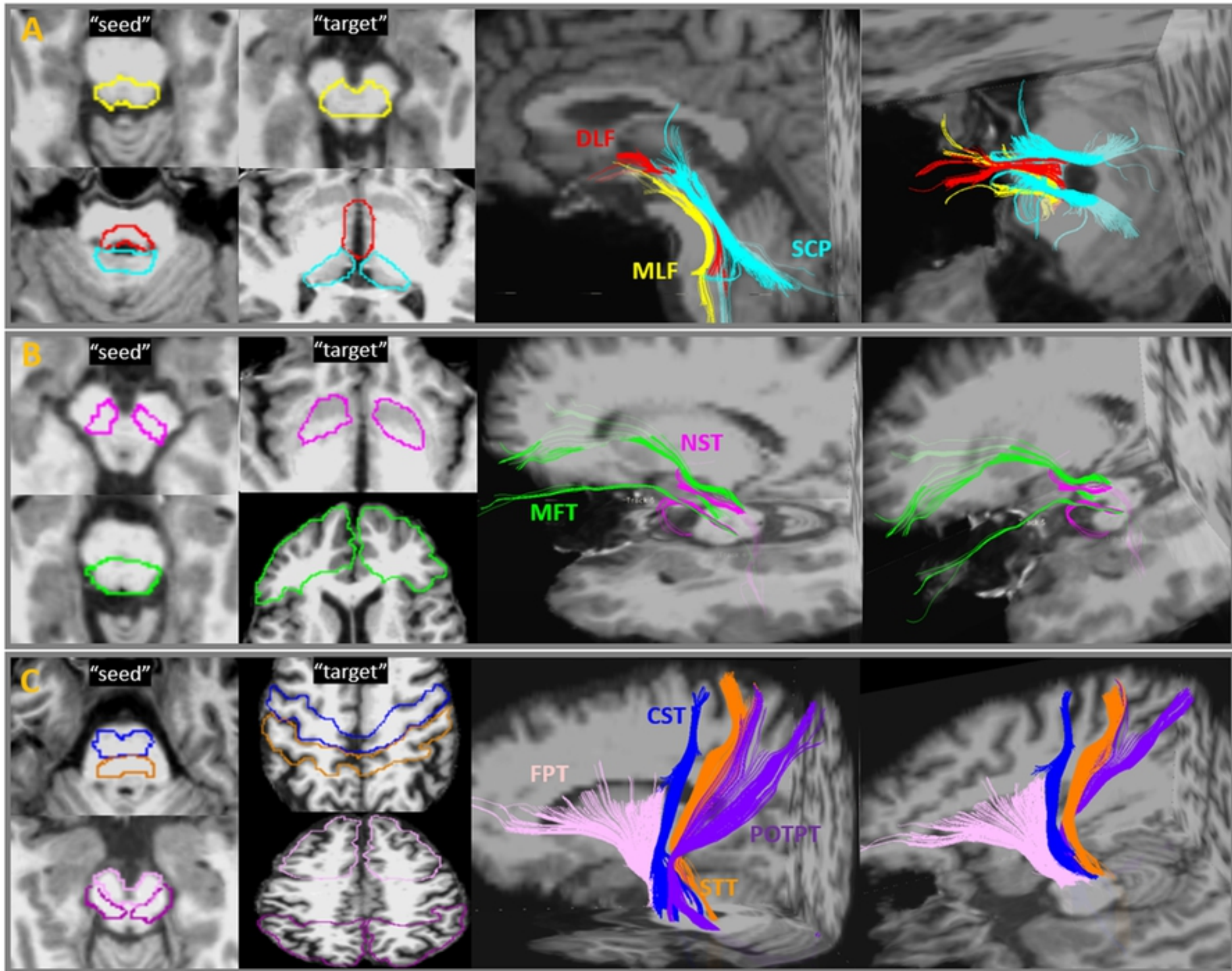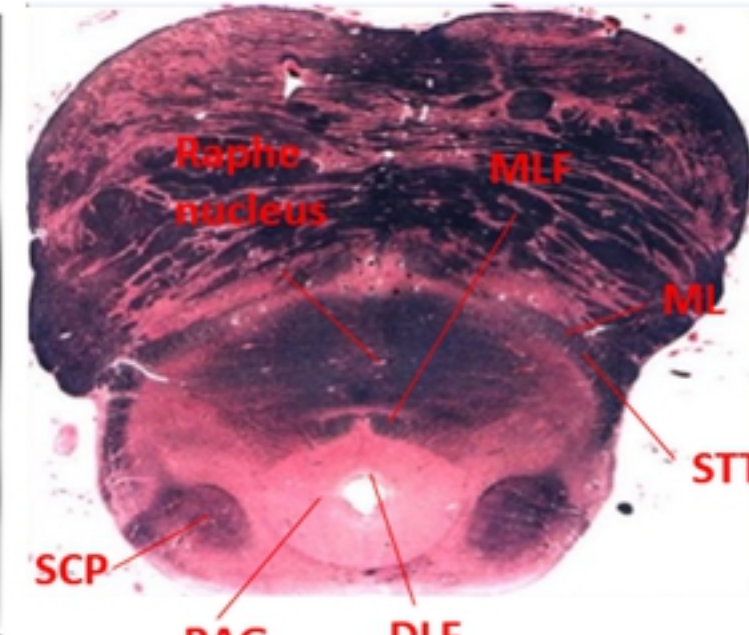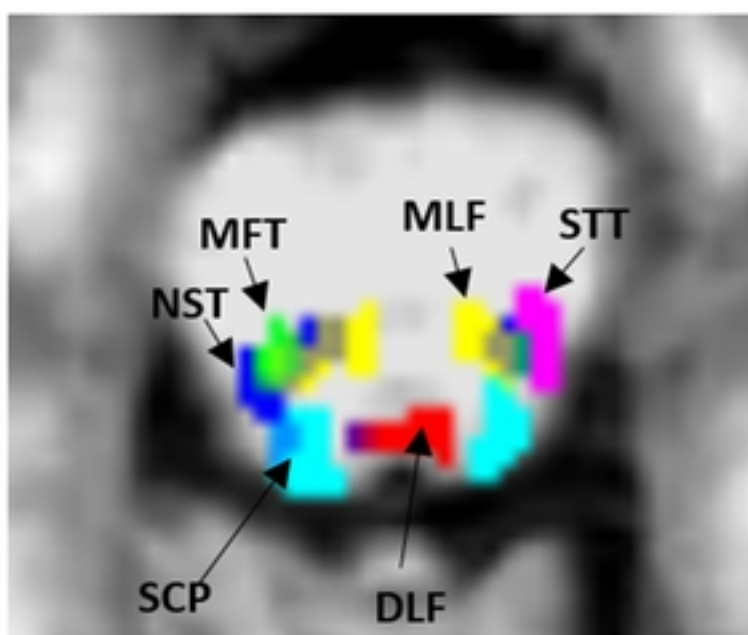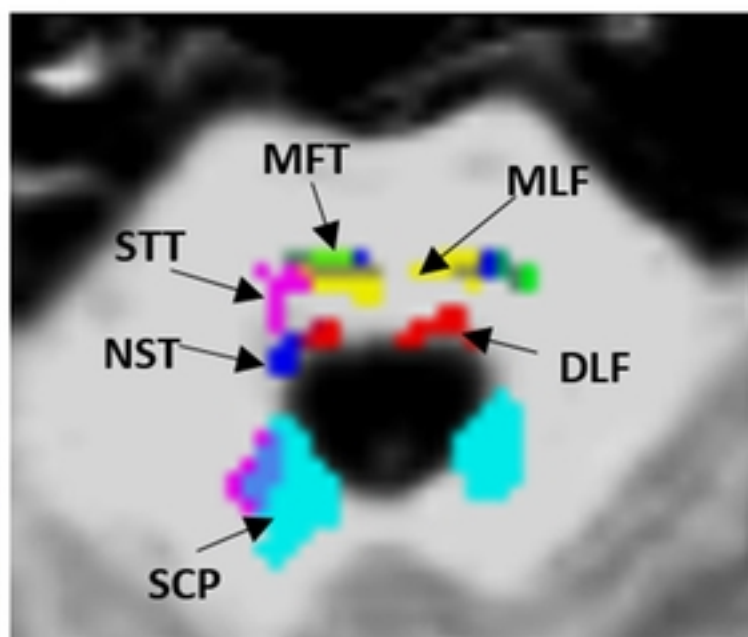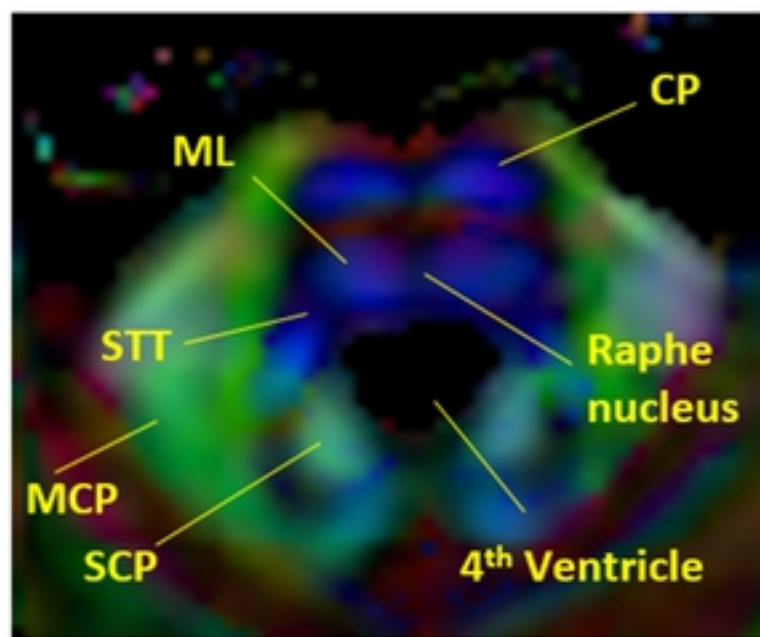
Figure 1

Figure 2
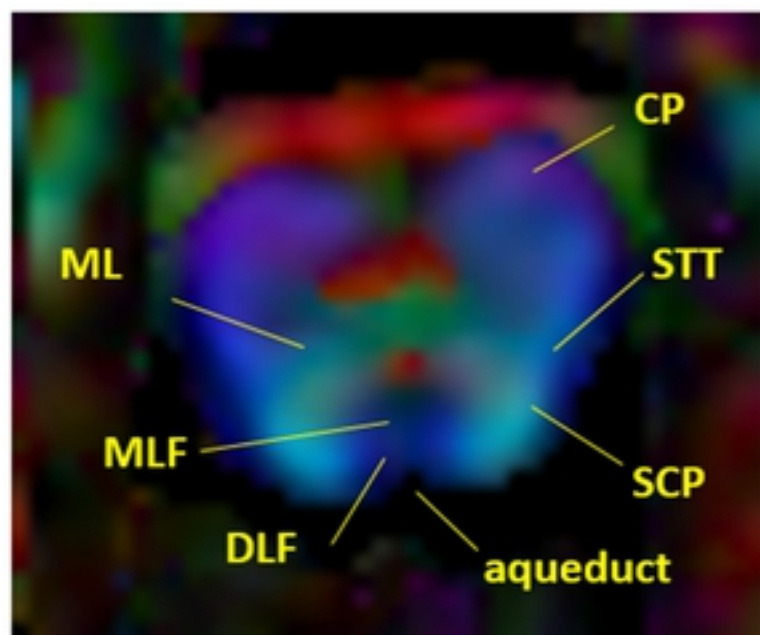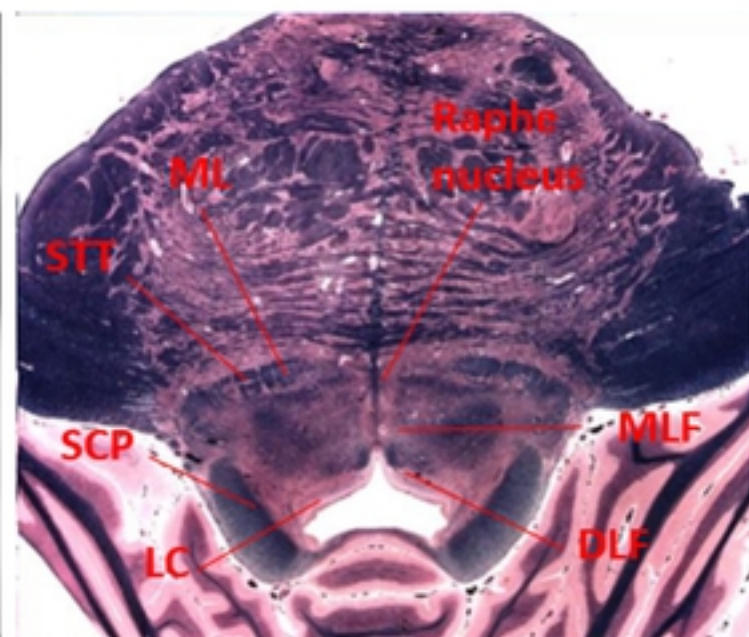
Figure 3

Figure 4

Figure 5