

The unresolved problem of synaptic connectivity in the context of the cerebro-cerebellar loop

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Synopsis

Recently advanced tractography has been used for assessing the feasibility of characterizing cerebro-cerebellar tracts, acknowledging the issue of how tractography deals with polysynaptic connectivity in particular through the thalamus. In this work, four different synaptic connectivity configurations at thalamic level were hypothesized and each one of them was reconstructed using high-quality HCP data. Our findings revealed the key role of thalamic connectivity for characterizing the cerebro-cerebellar connections. We can hypothesize that the relation between streamlines entering/outing the thalamic synapses is multiplicative and our findings using polysynaptic thalamic tracts support the fact that cognitive/associative areas are the mainly involved in the cerebro-cerebellar connections.

Purpose

The cerebellar involvement in both cognitive and motor functions is increasingly recognized(1). Furthermore, cerebellar processing is mediated by the cerebro-cerebellar loop composed of: cerebello-thalamo-cortical (CTC) and cortico-ponto-cerebellar pathways(2). Recent investigations using advanced MRI techniques assessed the feasibility of characterizing such tracts in humans in vivo(3-5), acknowledging, but not resolving the issue of how tractography deals with polysynaptic connectivity, e.g. through the thalamus. Current tractography algorithms use deep GM masks to stop connectivity and reconstructing tracts in a mono-synaptic fashion(6). Remains unresolved how to reconstruct loops that involve several relays and polysynaptic connectivity. Here, we present possible strategies to reconstruct synaptic connectivity in the particular case of the cerebro-cerebellar loop. We investigated how to reconstruct the efferent tract, which comes from the cerebellar cortex, through the superior cerebellar peduncle(SCP) and the red nucleus(RN), reaching the thalamus and continuing towards the cerebral cortex. We hypothesized four different synaptic connectivity configurations at thalamic level, developed a pipeline to reconstruct each one of them and characterize the CTC pathways starting from the high-quality data from the Human Connectome Project(7).

Methods

Subjects: 28 subjects (10/18 male/female; 22-35 years) were acquired using a Siemens 3T Connectome Skyra scanner. Minimal pre-processed 3DT1-weighted images and diffusion weighted data (1.25mm isotropic resolution, b=1000,2000,3000s/mm², 90 directions) were downloaded from ConnectomeDB(<http://db.humanconnectome.org>).

Whole-brain tractography: Fibre-orientation distributions (FODs) were calculated using the multi-shell-multi-tissue CSD(8). Whole-brain tractogram was performed using probabilistic streamline tractography (iFOD2)(9): step=1mm, angle=45°, FOD threshold=0.1, 30 million streamlines. 5-classes segmentation was performed on 3DT1-weighted images and WM-GM interface was used for randomly seeding the streamlines within the Anatomically-Constrained Tractography framework(6).

Cerebro-cerebellar connections: An ad-hoc atlas was created in MNI152 space combining deep GM structures, AAL(10) and SUIT atlases(11). Cerebral and cerebellar cortices were parcellated according to anatomical and functional basis(12). The atlas was dilated to overlap GM-WM interface and transformed to subject-space. Afferent and efferent possible cerebro-cerebellar connections were selected from the whole-brain tractogram as those connecting cerebral cortical and subcortical structures with contralateral cerebellar cortical areas.

CTC identification: Two steps were implemented: 1) Cerebellar peduncles masks were created using a cerebellar WM atlas(13). RN masks were created averaging hand-drawn masks on six randomly chosen subjects. From the identified cerebro-cerebellar connections, right/left monosynaptic CTC pathways were selected by isolating streamlines passing through the SCP and contralateral RN masks and avoiding streamlines passing through the other cerebellar peduncles. 2) Some of the streamlines from step 1) terminated in the thalamus. To identify the continuation of the efferent cerebellar pathway towards the cortex, the intersection between each thalamus and each CTC streamlines from step 1) was used as starting point to select streamlines originating in the thalamus and reaching the ipsilateral cerebral cortex. To investigate how thalamic polysynaptic tracts may affect the CTC pathway, three different hypotheses were considered for streamlines entering/outing the thalamus: i) one-to-one relationship; ii) one-to-n relationship, n=number of ongoing streamlines greater than 1; iii) one-to-all relationship. For each overall CTC pathway, we calculated the proportion of the tract projecting to a particular cortical ROI by assessing the $\text{trGM}_{\text{cROI}}$ index(3). One-way ANOVA was performed among $\text{trGM}_{\text{cROI}}$ of all tracts.

Results

Figure 1 shows both monosynaptic and polysynaptic CTC pathways reconstructed without (b) and with (c,d,e) adding thalamic streamlines, respectively. Table 1 and 2 report $\text{trGM}_{\text{cROI}}$ for all CTC pathways with respect to cerebral and cerebellar parcellations, respectively. It's worth noting that the inclusion of connections originated in the thalamic synapses affects cerebral indices while cerebellar indices aren't affected.

Discussion

Our main finding is confirming that thalamic connectivity has a fundamental influence on the characterization of the cerebro-cerebellar connections. From physiology, it is known that synapses amplify the number of entering connections, but it isn't clear which is this multiplication factor. Our results also confirm previous work(3) where the majority of the CTC streamlines projects to cerebellar cognitive/associative areas and aren't affected by thalamic synapses. If we assume that this proportion needs to be reflected also in the connectivity to the cerebral cortex, our results indicate that the most plausible option for the efferent thalamic projections are given by the one-to-n and the one-to-all strategies, which are not reconstructed by traditional tractography algorithms (Fig 1c,1d and Table 1). However, when considering all cortical parcellations, our work presents some differences to(3), such as in the occipital and temporal lobes. These discrepancies require further investigation as could be due to the improved image quality of the present dataset, to distortion correction and reduced partial volume effects. In conclusion, our work has highlighted possible ways to address the problem of poly-synapses in the

reconstruction of cerebral loops, which produce different results depending on the assumption on thalamic synapses connectivity. Further multi-level studies are warranted to identify the actual cerebro-cerebellar connectivity and propose algorithms able to solve this major issue.

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Figures

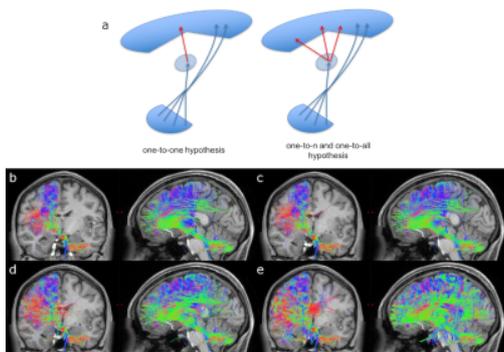


Figure 1: Schemes (a) and coronal/sagittal view of the left CTC pathways reconstructed using different hypothesis about streamlines entering and outing the thalamus. (b) monosynaptic CTC pathway without the contribution of thalamic connectivity; (c) polysynaptic CTC pathway with one-to-one thalamic connectivity; (d) polysynaptic CTC pathway with one-to-n thalamic connectivity; (e) polysynaptic CTC pathway with one-to-all thalamic connectivity.

| Cerebrum | CTC monosynaptic | CTC + one-by-one | CTC + one-by-n | CTC + one-by-all | ANOVA p-value |
|-----------------------|------------------|------------------|----------------|------------------|---------------|
| | mean(sd) | mean(sd) | mean(sd) | mean(sd) | |
| Frontal lobe | 60.0(6.2) | 53.8(6.1) | 43.6(5.2) | 34.5(3.7) | <0.001 |
| Prefrontal cortex | 9.0(3.6) | 7.7(2.8) | 7.0(1.9) | 7.1(1.3) | 0.013 |
| Limbic cortex | 8.8(2.0) | 8.7(1.8) | 10.1(1.9) | 11.3(1.3) | <0.001 |
| Parietal lobe | 18.5(5.6) | 24.4(5.3) | 27.7(3.0) | 26.7(1.8) | <0.001 |
| Temporal lobe | 1.5(0.9) | 2.5(1.0) | 5.4(2.0) | 10.3(2.2) | <0.001 |
| Occipital lobe | 2.1(1.0) | 3.0(1.1) | 6.2(2.0) | 10.1(2.0) | <0.001 |
| Motor area | 54.9(5.6) | 48.2(6.0) | 37.7(5.2) | 29.1(3.4) | <0.001 |
| Associative area | 33.2(4.8) | 35.8(4.7) | 43.2(4.6) | 49.4(3.0) | <0.001 |
| Primary somatosensory | 9.5(3.0) | 12.3(2.9) | 11.4(1.7) | 8.9(1.2) | <0.001 |
| Primary auditory | 0.3(0.3) | 0.6(0.4) | 1.5(0.7) | 2.5(0.8) | <0.001 |
| Visual cortex | 2.1(1.0) | 3.0(1.1) | 6.2(2.0) | 10.1(2.0) | <0.001 |

Table 1: $\text{trGM}_{\text{cROI}}$ values for anatomical and functional cerebral parcellations with corresponding statistical results among the four different CTC pathways. Data are expressed as Mean and standard deviation (SD). Significance was set at $p < 0.05$ (with correction).

| Cerebellum | CTC monosynaptic | CTC + one-by-one | CTC + one-by-n | CTC + one-by-all | ANOVA p-value |
|------------------------|------------------|------------------|----------------|------------------|---------------|
| | mean(sd) | mean(sd) | mean(sd) | mean(sd) | |
| Lobules I-V | 12.8(3.3) | 12.8(3.4) | 13.1(3.4) | 13.8(3.6) | ns |
| Lobule VI | 7.2(3.0) | 7.2(3.0) | 7.2(3.0) | 7.2(3.0) | ns |
| Crus / Lobule VIIa | 50.0(7.8) | 49.9(7.8) | 49.7(7.9) | 49.0(7.9) | ns |
| Lobules VIIb, VIII | 17.4(4.1) | 17.4(4.1) | 17.3(4.1) | 17.1(4.0) | ns |
| Lobules IX, X | 12.6(4.8) | 12.6(4.7) | 12.7(4.8) | 13.0(4.7) | ns |
| Primary motor area | 12.8(3.3) | 12.8(3.4) | 13.1(3.4) | 13.8(3.6) | ns |
| Cognitive/sensory area | 78.1(4.0) | 78.1(4.1) | 77.8(4.1) | 77.2(4.2) | ns |
| Sensory-motor area | 9.1(2.4) | 9.1(2.4) | 9.1(2.4) | 9.0(2.3) | ns |

Table 2: $\text{trGM}_{\text{cROI}}$ values for anatomical and functional cerebellar parcellations with corresponding statistical results among the four different CTC pathways. Data are expressed as Mean and standard deviation (SD). Significance was set at $p < 0.05$ (with correction).