# Brainstem structural connectivity changes in isolated REM sleep behavior disorder by 7 Tesla MRI

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## Objective

To investigate brainstem structural connectivity changes in isolated REM sleep behavior disorder (iRBD) patients using an in-vivo probabilistic brainstem nuclei atlas and 7 Tesla high angular resolution diffusion MR imaging.

## Background

iRBD is characterized by the absence of REM-sleep muscle atonia. iRBD patients have up to 73.5% risk of developing a neurodegenerative synucleinopathy after 12 years from the iRBD-diagnosis[1]. Brainstem pathophysiology underlying iRBD has been described in animal models, yet it is understudied in living humans due to the lack of an in-vivo brainstem nuclei atlas and to the limited sensitivity of conventional MRI.

## Methods

Data acquisition: We performed 7 Tesla MRI [table1] in 12 iRBD patients (age: 67.9±1.7 yrs) and 12 controls (age: 66.3±1.6 yrs), under IRB-approval. Analysis: *a) Preprocessing:* see Table 1. *b) Definition of seed and target regions for DTI-based connectivity analysis:* see Table 2 and Figure 1. *c) Single-subject and group DTI-based connectivity analysis:* We run probabilistic tractography and computed a "structural-connectivity-index" for each pair of seed-target masks (= fraction of streamlines propagated from seed reaching target). *d) Statistical analysis:* Wilcoxon test was used to compare the differences between groups.

**Table 1. 7 T MRI acquisition parameters**

| Acquisition Parameters | MEMPRAGE | DWI |
|---|---|---|
| Repetition-time (TR) | 2.53 s | 7.4 s |
| Echo-time/s (TE) | 2.39/5.62 ms | 66.8 ms |
| Inversion-time | 1.1s | - |
| Isotropic spatial resolution | 0.75 mm | 1.7 mm |
| Bandwidth | 332Hz/pixel | 1456 Hz/pixel |
| Partial-Fourier | None | 6/8 |
| PE-direction | "A-P" | "A-P" |
| Diffusion scheme | - | unipolar |
| Number of diffusion-directions | - | 60 |
| b-value | - | 2500 s/mm$^2$ |
| b0 images | - | 7 interspersed "A-P"<br>7 interspersed "P-A" |
| Acquisition-time | 6'34'' | 8'53'' |

**Table 1.** To compute a structural connectome of brainstem nuclei we acquired diffusion weighted images (DWI). Further, to aid the coregistration of DWI to stereotactic (MNI) space we also acquired a T1-weighted MEMPRAGE image. ***Preprocessing:*** We parcellated the root-mean-square MEMPRAGE image with Freesurfer. DTIs were de-noised, motion and distortion-corrected, and computed the fractional anisotropy (FA) and S0. To map Freesurfer parcellation to native DTI-space, we computed an affine boundary-based transformation between the MEMPRAGE and S0 images. To map the brainstem nuclei atlas [2–4] to native DTI-space, we computed the bivariate diffeomorphic transformations between IIT-MNI FA/S0 templates [5] and single-subject FA/S0 images.

**Table 2. List of brainstem nuclei relevant for iRBD/premanifest-synucleinopathy** (according to the Braak hypothesis[6]). These nuclei are involved in motor and/or arousal functions (as marked in the appropriate columns).

| Nuclei name | Nuclei acronym | Braak Hypothesis | RBD | Motor | Arousal |
|---|---|---|---|---|---|
| **1. Substantia nigra pars reticulata** | SN1 (SNR) | X (stage 3) | X | X | X |
| **2. Substantia nigra pars compacta** | SN2 (SNC) | X (stage 3) | X | X | X |
| **3. Caudal linear raphe** | CLi | X (stage 3) | X | | X |
| **4. Pedunculotegmental nucleus** | PTg | X (stage 2-3) | X | X | X |
| **5. Paramedian raphe** | PMnR | X (stage 2-3) | X | | X |
| **6. Dorsal raphe** | DR | X (stage 2-3) | X | | X |
| **7. Median raphe** | MnR | X (stage 2-3) | X | | X |
| **8. Raphe magnus** | RMg | X (stage 2) | X | | X |
| **9. Raphe obscurus** | ROb | X (stage 2) | | X | |
| **10. Raphe pallidus** | RPa | X (stage 2) | | X | |
| **11-12. Locus coeruleus, subcoeruleus** | LC, SubC | X (stage 2) | X | | X |
| **13-15. Medullary reticular formation** | iMRt, sMRt, PCRtA | X (stage 1-2) | X | X | X |
| **16. Dorsal motor nucleus of the vagal nerve** | VSM (DMVN) | X (stage 1) | | X | |
| **17. Laterodorsal tegmental nucleus** | LDTg(-CGPn) | | X | | X |
| **18. Pontine reticular nucleus, oral part** | PnO(-PnC) | X | X | X | X |
| **19. Cuneiform nucleus** | CnF | | | X | X |
| **20. Periaqueductal gray** | PAG | X | X | | X |

## Results

The structural connectome of brainstem nuclei relevant for iRBD/premanifest-synucleinopathy showed connectivity changes (specifically in 14 out of 32 brainstem seeds) across groups ($Z = 2.6$, $p < 0.01$) mainly within brainstem nuclei (Figure 2). Specifically, we found impaired connectivity in iRBD between REM-on and REM-sleep muscle-atonia medullary areas. This is in agreement with animal studies showing decreased excitatory connectivity influences between REM-on regions and ventro-medullary nuclei, the latter projecting to spinal motoneurons critical for generating muscle atonia during REM-sleep[7]. Most of REM-off areas did not show differences in connectivity between groups. Interestingly, ponto-medullary brainstem nuclei, known to be involved in REM atonia, showed decreased structural inter-connectivity, possibly related to an underlying neurodegeneration process. In contrast, meso-pontine regions showed overall increased inter-connectivity.

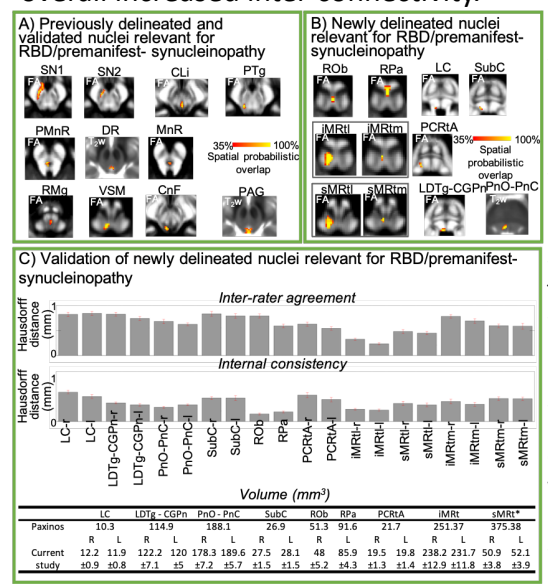
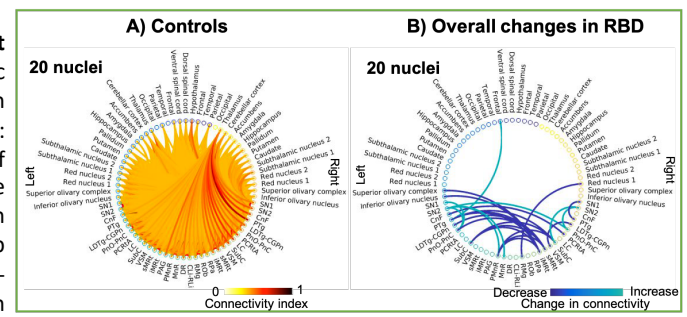



**Figure 1. Definition of seed and target regions.** As *seed regions*, we used structural probabilistic atlas labels of 20 brainstem nuclei relevant for iRBD/premanifest-synucleinopathy. **A)** Probabilistic atlas labels of 11 brainstem nuclei relevant for iRBD/premanifest-synucleinopathy, previously published[2–4] used as seeds and brainstem targets in this study. **B)** Additional probabilistic atlas labels of nine nuclei relevant for iRBD/premanifest-synucleinopathy generated in the current work (11 nuclei are shown because two nuclei, iMRt and sMRt, displayed two subregions, a lateral larger subregion – l – and a medial smaller subregion – m). Very good (i.e., up to 100%) spatial agreement of labels across subjects was observed indicating the feasibility of delineating the probabilistic label of these nuclei. As *target regions*, we used the probabilistic atlas labels of these 20 brainstem nuclei in addition to superior olivary complex, inferior olivary nucleus, red nucleus, subthalamic nucleus, hypothalamus, cortical/subcortical bilateral regions obtained in each subject from the MEMPRAGE Freesurfer-parcellation and ventral/dorsal spinal cord. **C)** Validation of the nuclei displayed in B). The nuclei volume from the literature[8] and the volume (mean ± SE across subjects) of each final label in native space are shown.

**Figure 2. Structural connectome of brainstem nuclei relevant for iRBD/premanifest-synucleinopathy.** For the probabilistic tractography we propagated 100,000 streamlines from each seed and computed a "structural-connectivity-index" (range: [0 1]) for each pair of seed-target masks (= fraction of streamlines propagated from seed reaching target). We averaged the connectivity-index of brainstem nuclei with target-regions across subjects and displayed the group connectome with a 2D circular diagram. Wilcoxon test (two-sided, $p < 0.01$) was used to compare the differences in connectivity indices between groups. **A)** Structural tractography-based connectome of 20 brainstem nuclei relevant for iRBD (list of nuclei shown in figure 1A) in controls (average connectivity index across 12 controls displayed). **B)** Statistically significant differences in structural connectivity between iRBD patients and controls (Wilcoxon test, $p < 0.01$, *n = 12*); note that, except for a significant link with the frontal cortex, alterations of connectivity pathways in iRBD occurred exclusively within brainstem nuclei.




## Conclusions

Decreased structural connectivity between REM-on and medullary brainstem nuclei underlies REM-sleep muscle atonia in iRBD patients.

## References

[1] Postuma et al, Brain, 2019.
[2] Bianciardi et al, Brain Connect, 2015.
[3] Bianciardi et al, Neuroimage, 2018.
[4] Singh et al, Front Neurosc, 2020.
[5] Avants BB et al, Neuroimage, 2011.
[6] Braak et al, Cell Tissue Res, 2004.
[7] Valencia Garcia et al, Nat Commun, 2018.
[8] Paxinos et al, Organization of brainstem nuclei, 2012.