Title: 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.

Methods: Data acquisition: We performed 7 Tesla MRI, under IRB-approval, in 12 iRBD patients (age: 68±1.6yrs) and 12 controls (age: 66.3±1.6yrs). We acquired 0.75mm-isotropic T_1-weighted *MEMPRAGE* and 1.7mm-isotropic *spin-echo diffusion-weighted MRI*.
Data analysis: *a) Preprocessing:* We parcellated the root-mean-square MEMPRAGE image with Freesurfer. *b) Definition of seed and target regions:* We used as seeds the structural probabilistic atlas labels of 20 brainstem nuclei relevant for RBD[1-3] and as targets 197 cortical/subcortical regions (Freesurfer parcellations, brainstem nuclei[1-3] and spinal cord). *c) DTI-based connectivity analysis:* We performed probabilistic-tractography (iFOD2-MRtrix3) propagating 100,000 streamlines from each seed. We computed a "structural-connectivity-index" for each pair of seed-target masks (= fraction of streamlines propagated from seed reaching target). *d) Statistical analysis:* We averaged the "structural-connectivity-index" across subjects and Wilcoxon test was used to compare the differences between groups.

Results/Discussion: The structural connectome of brainstem nuclei relevant for RBD showed cross-sectional connectivity changes of 14 brainstem seeds mainly within the brainstem (Figure 1). We found impaired connectivity in iRBD between REM-on and REM-sleep muscle-atonia medullary areas[4] (Figure 2). This agrees with animal studies showing decreased excitatory connectivity between REM-on regions and ventro-medullary nuclei, the latter projecting to spinal motoneurons, which generate muscle atonia during REM-sleep[5]. 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, possibly indicating compensatory mechanisms.

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

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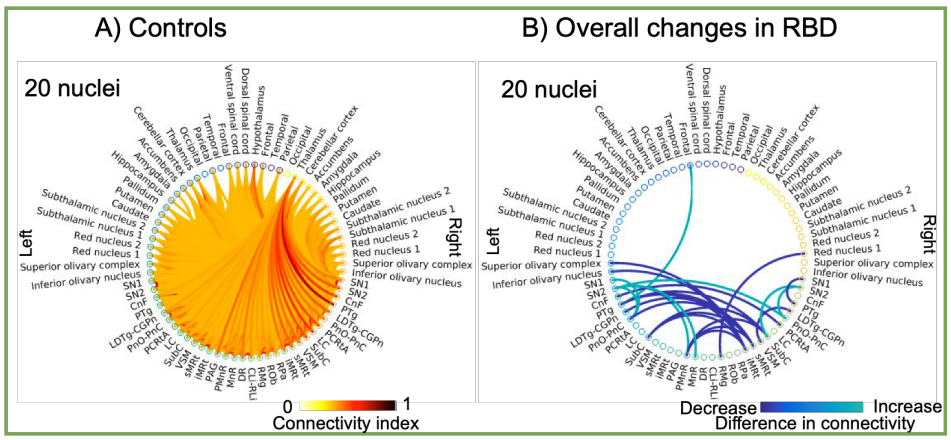

Figure 1. A) Structural tractography-based connectome of 20 brainstem nuclei relevant for RBD in controls (average structural-connectivity-index across 12 controls displayed). **B)** Statistically significant differences in structural connectivity between iRBD patients and controls (Wilcoxon test, $p < 0.01$, $n = 12$); note that, except for a significant link with the frontal cortex, alterations of connectivity pathways in iRBD occurred exclusively within brainstem nuclei. List of nuclei abbreviations: substantia nigra-subregion1 (SN1), substantia nigra-subregion2 (SN2), cuneiform nucleus (CnF), pedunculotegmental nucleus (PTg), laterodorsal tegmental nucleus- central gray of the rhombencephalon (LDTg-CGPn), pontine reticular nucleus oral part- pontine reticular nucleus caudal part (PnO-PnC), parvocellular reticular nucleus alpha part (PCRtA), locus coeruleus (LC), subcoeruleus nucleus (SubC), viscero-sensory motor nuclei complex (VSM), superior medullary reticular formation (sMRt), inferior medullary reticular formation (iMRt), periaqueductal gray (PAG), paramedian raphe nucleus (PMnR), median raphe nucleus (MnR), dorsal raphe (DR), caudal-rostral linear raphe (CLi-RLi), raphe magnus (RMg), raphe obscurus (ROb) and raphe pallidus (RPa).

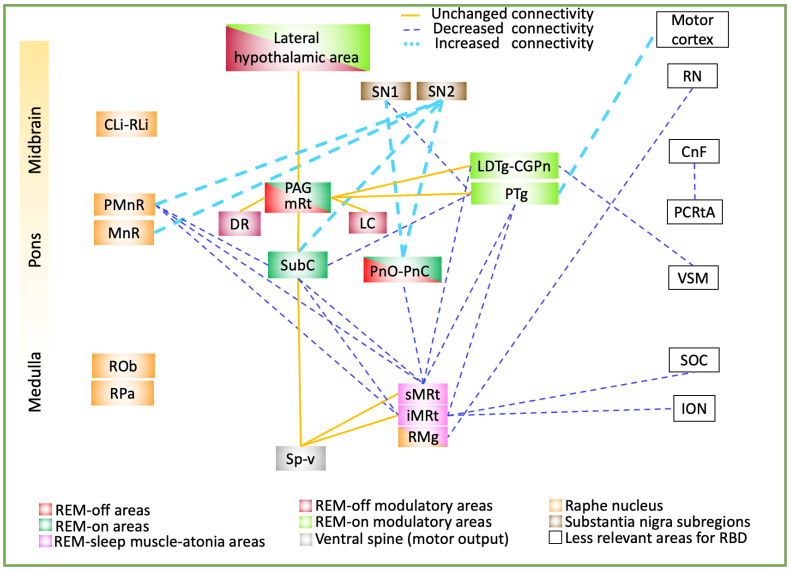

Figure 2. Diagram summarizing the structural connectivity changes in RBD human subjects in the present study. The RBD relevant nuclei/regions were color-coded based on their functions as previously reported.[2] Notably, decreased structural connectivity mainly occurred in ponto-medullary brainstem nuclei previously postulated to be involved in REM atonia, while increased connectivity was found in meso-pontine brainstem nuclei. Moreover, the direct pathway between SubC and the spinal cord was preserved, while the indirect pathway through the medullary reticular formation (sMRt, iMRt) displayed decreased connectivity.