

## Supplementary Files

**Supplementary Table 1: Tractograms generated using MRtrix.** All probabilistic tractographies were performed unidirectionally, with minimum length of 10 mm and maximum length of 250 mm. We used exclusion criteria by means of tailored region of interest (ROI) that were created using the Harvard-Oxford cortical and structural atlases for cerebral cortex and subcortical structures [17], and the probabilistic atlas of the human cerebellum for cerebellar cortex [18], both distributed with FMRIB Software Library (FSL). The following 14 tracts were reconstructed: ventral tegmental area (VTA)-Nucleus accumbens; VTA-amygdala; VTA-hippocampus; VTA-dorsolateral(dl) prefrontal cortex (PFC); VTA-medial orbitofrontal(mOf)PFC; VTA-lateral orbitofrontal(LOf)PFC; locus coeruleus (LC)-dlPFC; LC-mOfPFC; LC-IOfPFC; median raphe (M)R-Cerebellar Lobule V-VI; MR-Cerebellar Lobule VII; dorsal raphe (DR)-Cerebellar lobule V-VI; DR-Cerebellar lobule VII; cingulum. Averaged FD, FC, and FDC were calculated across the fixels in each tract in MS patients and HC.

Tract	Angular threshold	Number of streamlines	Exclusion criteria
VTA-N <sub>acc</sub>	20°	100	PFC, PCC, corpus callosum
VTA-amygdala	20°	100	Temporal lobe, hippocampus
VTA-hippocampus	20°	100	Temporal lobe, amygdala
VTA-dlPFC	20°	1000	IOfPFC, mOfPFC, ACC, corpus callosum, temporal and parietal lobes, thalamus
VTA-mOfPFC	20°	500	IOfPFC, dlPFC, ACC, temporal and parietal lobes, thalamus, N <sub>acc</sub>
VTA-IOfPFC	20°	500	mOfPFC, dlPFC, temporal lobe, parietal lobe, thalamus, N <sub>acc</sub>
LC-dlPFC	20°	1000	IOfPFC, mOfPFC, ACC, corpus callosum, temporal and parietal lobes, thalamus
LC-mOfPFC	20°	500	IOfPFC, dlPFC, ACC, temporal and parietal lobes, thalamus, N <sub>acc</sub>
LC-IOfPFC	20°	500	mOfPFC, dlPFC, temporal and parietal lobes, thalamus, N <sub>acc</sub>
MR-Cer Lob VII	10°	1000	/

MR-Cer Lob V+VI	10°	1000	Cer Lob VII
DR-Cer Lob VII	10°	1000	/
DR-Cer Lob V+VI	10°	1000	Cer Lob VII
Cingulum	20°	10.000	Temporal lobe, hippocampus, corpus callosum

Abbreviations = VTA: ventral tegmental area; N<sub>acc</sub>: nucleus accumbens; dlPFC: dorso-lateral prefrontal cortex; mOfPFC: medial orbito-frontal prefrontal cortex; lOfPFC: lateral orbito-frontal prefrontal cortex; PCC: posterior cingulate, cortex; ACC: anterior cingulate cortex; LC: locus coeruleus; MR: median raphe; DR: dorsal raphe; Cer Lob: Cerebellar Lobule.

**Supplementary Table 2: Correlations between fibre density and cross-section (FDC) and fatigue scores.** Correlations between FDC in the selected fibre tracts in relapsing-remitting multiple sclerosis patients and fatigue scores, as computed by the total score of the Modified Fatigue Impact Scale (MFIS-Tot), which is obtained by adding the scores of the MFIS subscales: cognitive (MFIS-Cog), physical (MFIS-Phys) and psychosocial (*not shown*). Correlations between FDC and MFIS scores were performed assessing the Pearson correlation coefficient (*r*). Statistical threshold was set to  $p < 0.025$  after Bonferroni correction for multiple comparisons.

Tract	MFIS-Tot		MFIS-Cog		MFIS-Phys	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
<b>FDC VTA-N<sub>acc</sub></b>	-0,19	0,126	<b>-0,28</b>	<b>0,021</b>	0,14	0,248
<b>FDC VTA-amygdala</b>	-0,21	0,090	<b>-0,27</b>	<b>0,017</b>	-0,03	0,796
<b>FDC VTA-hippocampus</b>	<b>-0,32</b>	<b>0,008</b>	<b>-0,36</b>	<b>0,002</b>	-0,05	0,662
<b>FDC VTA-dlPFC</b>	-0,15	0,237	-0,25	0,037	0,09	0,475
<b>FDC VTA-mOfPFC</b>	-0,14	0,272	-0,25	0,040	0,06	0,610
<b>FDC VTA-IOfPFC</b>	-0,16	0,185	<b>-0,30</b>	<b>0,014</b>	0,05	0,688
<b>FDC LC-dlPFC</b>	-0,16	0,180	<b>-0,29</b>	<b>0,017</b>	0,07	0,585
<b>FDC LC-mOfPFC</b>	-0,15	0,219	-0,26	0,031	0,04	0,773
<b>FDC LC-IOfPFC</b>	-0,15	0,219	<b>-0,28</b>	<b>0,019</b>	0,06	0,601
<b>FDC MR-Cer Lob VII</b>	-0,17	0,164	-0,27	0,025	0,21	0,084
<b>FDC MR-Cer Lob V+VI</b>	-0,13	0,275	-0,22	0,068	0,17	0,173
<b>FDC DR-Cer Lob VII</b>	-0,10	0,419	-0,14	0,244	0,18	0,146
<b>FDC DR-Cer Lob V+VI</b>	-0,03	0,818	-0,07	0,569	0,24	0,052
<b>Cingulum</b>	-0,22	0,066	<b>-0,34</b>	<b>0,004</b>	0,09	0,471

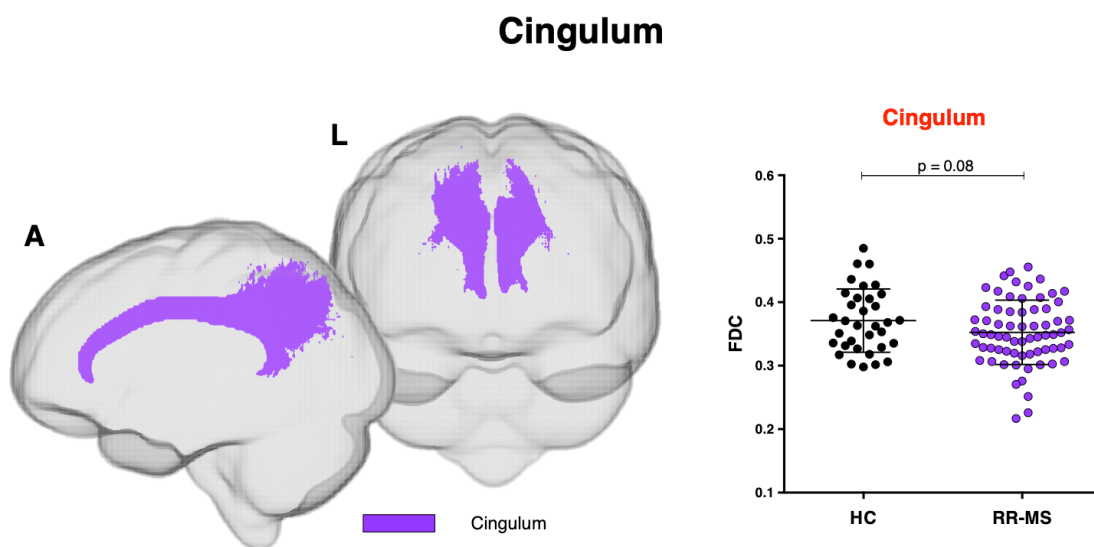
[Abbreviations: MFIS: Modified Fatigue Impact Scale; Tot: total score; Cog: cognitive subscale; Phys: physical subscale; FDC: fibre density and cross-section; VTA: ventral tegmental area; N<sub>acc</sub>: nucleus accumbens; dlPFC: dorsolateral prefrontal cortex; mOfPFC: medial orbito-frontal prefrontal cortex; IOfPFC: lateral orbito-frontal prefrontal cortex; LC: locus coeruleus; Cer Lob: Cerebellar Lobule; MR: median raphe; DR: dorsal raphe]

**Supplementary Table 3: Partial correlations between fibre density and cross-section (FDC) and fatigue scores, controlling for depression and sleep disturbances.** Partial correlations (controlled for the scores of Hospital Anxiety and Depression Scale [HADS-D] and the Epworth Sleepiness Scale [ESS]) between FDC in the selected fibre tracts in relapsing-remitting multiple sclerosis patients and fatigue scores, as computed by the total score of the Modified Fatigue Impact Scale (MFIS-Tot), which is obtained by adding the scores of the MFIS subscales: cognitive (MFIS-Cog), physical (MFIS-Phys) and psychosocial (*not shown*). Partial correlations between FDC and MFIS scores were performed assessing the Pearson correlation coefficient ( $r$ ), controlling for HADS-D and ESS. Statistical threshold was set to  $p < 0.025$  after Bonferroni correction for multiple comparisons.

Tract	MFIS-Tot		MFIS-Cog		MFIS-Phys	
	$r$	$p$	$r$	$p$	$r$	$p$
<b>FDC VTA-Nacc</b>	-0,19	0,121	<b>-0,27</b>	<b>0,025</b>	0,08	0,530
<b>FDC VTA-amygdala</b>	-0,21	0,088	<b>-0,28</b>	<b>0,021</b>	-0,09	0,447
<b>FDC VTA-hippocampus</b>	<b>-0,32</b>	<b>0,008</b>	<b>-0,36</b>	<b>0,002</b>	-0,07	0,587
<b>FDC VTA-dIPFC</b>	-0,15	0,240	-0,24	0,055	0,00	0,990
<b>FDC VTA-mOfPFC</b>	-0,14	0,271	-0,24	0,054	-0,01	0,923
<b>FDC VTA-IOfPFC</b>	-0,17	0,179	<b>-0,29</b>	<b>0,020</b>	-0,03	0,776
<b>FDC LC-dIPFC</b>	-0,17	0,170	<b>-0,28</b>	<b>0,022</b>	-0,02	0,867
<b>FDC LC-mOfPFC</b>	-0,16	0,198	-0,26	0,033	-0,02	0,855
<b>FDC LC-IOfPFC</b>	-0,16	0,204	<b>-0,28</b>	<b>0,023</b>	-0,02	0,877
<b>FDC MR-Cer Lob VII</b>	-0,17	0,168	-0,26	0,040	0,13	0,297
<b>FDC MR-Cer Lob V+VI</b>	-0,13	0,276	-0,21	0,091	0,10	0,400
<b>FDC DR-Cer Lob VII</b>	-0,09	0,444	-0,11	0,356	0,09	0,466
<b>FDC DR-Cer Lob V+VI</b>	-0,02	0,851	-0,06	0,711	0,18	0,145
<b>Cingulum</b>	-0,23	0,067	<b>-0,33</b>	<b>0,006</b>	0,03	0,791

[Abbreviations: MFIS: Modified Fatigue Impact Scale; Tot: total score; Cog: cognitive subscale; Phys: physical subscale; FDC: fibre density and cross-section; VTA: ventral tegmental area; N<sub>acc</sub>: nucleus accumbens; dlPFC: dorsolateral prefrontal cortex; mOfPFC: medial orbito-frontal prefrontal cortex; lOfPFC: lateral orbito-frontal prefrontal cortex; LC: locus coeruleus; Cer Lob: Cerebellar Lobule; MR: median raphe; DR: dorsal raphe]

**Supplementary Fig.1: Cingulum fibre tract-specific significant FDC decreases in patients with multiple sclerosis (RR-MS) compared to healthy control (HC).** The cingulum – connecting the anterior cingulate cortex with posterior cingulate cortex – is shown in glass brain representations using the *mrview* tool in MRtrix3. Differences in average FDC between HC and RR-MS patients in the cingulum is shown in scatter plots. Comparisons were tested by independent sample t-tests. The statistical threshold was set to  $p < 0.003$  after Bonferroni correction for multiple comparisons (FDC = fibre-density cross-section, A = anterior, L = left, R = right).



**Supplementary Figure 2: Differences in regional grey matter atrophy between MS patients and healthy controls.** Voxel-based morphometry analysis displayed significant grey matter loss in bilateral thalamus in MS patients, as compared to healthy controls. Results are reported at  $p_{FWE} < 0.05$ , corrected at peak level and are overlaid on the MNI\_T1 template using the *FSLeyes* tool in FSL (P = posterior, R = right).

