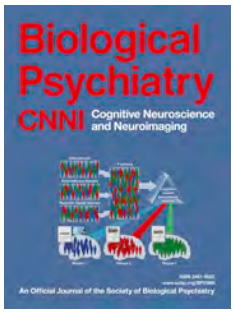

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Modulation of cerebellar-cortical connectivity induced by modafinil and its relationship with receptor and transporter expression

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Abstract

Background: Modafinil is primarily employed to treat narcolepsy but also as an off-label cognitive enhancer. Functional Magnetic Resonance Imaging (fMRI) studies indicate that modafinil modulates the connectivity of neocortical networks primarily involved in attention and executive functions. However, much less is known about the drug's effects on subcortical structures. Following preliminary findings, we evaluated modafinil's activity on the connectivity of distinct cerebellar regions with the neocortex. We assessed the spatial relationship of these effects with the expression of neurotransmitter receptors/transporters.

Methods: Patterns of resting-state fMRI (rs-fMRI) connectivity were estimated in 50 participants from scans acquired pre- and post-administration of a single (100 mg) dose of modafinil (n=25) or placebo (n=25). Using specific cerebellar regions as seeds for voxel-wise analyses, we examined modafinil's modulation on cerebellar-neocortical connectivity. Next, we conducted a quantitative evaluation of the spatial overlap between the modulation of cerebellar-neocortical connectivity and the expression of neurotransmitter receptors/transporters obtained by publicly available databases.

Results: Modafinil increased the connectivity of Crus I and Vermis IX with prefrontal regions. Crus I connectivity changes were associated with the expression of dopaminergic D_2 receptors. The Vermis I-II showed enhanced coupling with the dorsal anterior cingulate cortex and matched the expression of histaminergic H_3 receptors. The Vermis VII-VIII displayed increased connectivity with the visual cortex, an activity associated with dopaminergic and histaminergic neurotransmission.

Conclusion: Our study reveals modafinil's modulatory effects on cerebellar-neocortical connectivity. The modulation mainly involves Crus I and the Vermis and spatially overlaps the distribution of dopaminergic and histaminergic receptors and serotonin transporters.

Keywords: Modafinil; cerebellum; fMRI; receptors; crus; vermis.

Running title: Modafinil shapes the cerebellar-cortical connections

Introduction

Modafinil (2-diphenyl-methyl-sulphinil-2-acetamide) is a stimulant-like medication primarily used to treat narcolepsy (1). However, the compound has off-label applications that enhance attention, memory, and executive functions and increase alertness and response accuracy (1). The use of modafinil is attracting increasing interest as it may assist in maintaining optimal brain function or compensating for subtle, subclinical deficits linked to brain aging or early-stage dementia (2). At the same time, it is crucial to understand how this compound interacts with brain physiology to exert its positive effects on cognition.

The underlying mechanisms of action are still not completely understood and appear somewhat unspecific, as modafinil can boost the release of numerous neurotransmitters (3, 4). Modafinil directly binds the dopamine (DAT) and norepinephrine (NET) transporters, which it inhibits at modest potency. Although there is no direct evidence of modafinil binding to dopaminergic receptors (5), some studies have suggested that dopaminergic D_1 and D_2 are essential for the wakefulness induced by modafinil (4, 6) as well as the inhibitory action on transporters promotes an increase in extracellular dopamine concentrations, thereby favoring an indirect modulation of D_1 and D_2 receptor activity. In addition, modafinil administration significantly elevates extracellular levels of serotonin (5-HT), orexin, and histamine secondary to catecholamine effects. Finally, an effect on extracellular glutamate and GABA (except for the hypothalamus) is observed at higher doses (4).

At a system level, functional Magnetic Resonance Imaging (fMRI) plays a crucial role in investigating how drugs influence the brain's functional architecture, identifying brain regions whose activity is particularly impacted by the compound (7-9). Task-evoked fMRI is a powerful tool for exploring the effect of the drug on the brain's response to behavioral demands. However, this technique, dependent on the selection of specific tasks, does not provide a holistic exploration of the physiological effects, given that task-evoked activity is associated with only a small part (less than 5%) of the total brain energy consumption (10). Alternatively, the study of the spontaneous low-frequency fluctuations in the Blood Oxygenation Level Dependent (BOLD) signal at rest allows us to assess the drug's effects on the brain's physiological homeostatic processes, which explain a more significant portion of the brain energy consumption, providing insights on how the functional connectome is organized at rest to eventually support task-evoked activities (11). Rs-fMRI studies have revealed significant changes in connectivity induced by modafinil within and between brain networks (12-14). Much of the available evidence comes from studies focusing on the neocortex. In this context, we have shown that a single dose of modafinil in healthy young subjects increases the functional connection strength across task-positive networks, including the so-called Fronto-Parietal Control Network (FPCN), Dorsal Attention Network (DAN), and Salience/Ventral Attention Network (SVAN) (13-15). More recent studies (14) have provided further support to these findings, showing that modafinil enhances the physiological inverse coupling between task-positive networks, particularly the FPCN, and task-negative networks, such as the Default Mode Network (DMN) (12).

Much less is known about the effect of modafinil on subcortical-cortical connectivity, although the recognition of the contribution of subcortical regions in shaping the levels of consciousness, alertness, and attention is well established (16). Interestingly, an exploratory graph-based analysis from our laboratory (17) highlighted the effect of modafinil on the connectivity between the cerebellar and the visual cortex. Indeed, the evidence for an effect on the cerebellum is particularly intriguing from a neural standpoint, given the region's role in modulating cognitive functions (18, 19). Specifically, the corticocerebellar polysynaptic circuit forms a feedback loop that connects the neocortex and the cerebellum bidirectionally (20, 21). The anterior cerebellum is primarily linked to sensory and motor cortices, while the posterior cerebellum has stronger connections with association cortices (22). The interaction between the posterior cerebellum and the prefrontal and posterior parietal cortices is of particular interest, as these two cortical regions play a crucial role in modulating higher cognitive functions such as attention and executive control (23, 24). However, the cerebellum is not a homogeneous structure but consists of several regions that interact differently with the cortex to modulate neural processes (25, 26). Normative studies have shown that some regions exhibit preferential connectivity with distinct association networks (27, 28). Therefore, a comprehensive analysis of the cerebellar-cortical connectivity is needed to assess its anatomic-functional specificity.

One obvious question is whether modafinil modifies the connectivity of cerebellar regions more closely related to higher cognitive functions, such as the Crus I/II, based on its known coupling with DMN and FPCN. Moreover, it is still unclear whether physiological effects, measured through indices of neurovascular coupling, are related to the pharmacological mechanisms underlying the effect of modafinil. One way to link these two levels of explanation is to examine the spatial overlap between the modulation of functional connectivity and the topography of distinct neurotransmitter receptors/transporters. The maps of these proteins are available through existing databases (7). Based on receptor/transporter-binding profiles of modafinil at low to moderate doses (4), the analysis focuses on the serotoninergic (transporter: 5-HTT), dopaminergic (receptors: D_1, D_2; transporter: DAT), noradrenergic (transporter: NET), and histaminergic (receptor: H_3) neurotransmission. Given modafinil's established direct actions on DAT and NET, as well as its effects on the dopaminergic system, we anticipate that the expression maps of these transporters and D_1/D_2 receptors could show significant correlations with rs-fMRI maps. Additionally, considering that histamine is a key neurotransmitter regulating the sleep-wake cycle (29), one of modafinil's primary targets, we explored the potential involvement of H_3 receptors (30), crucial modulators of cerebral histaminergic activity. This investigation is supported by findings that modafinil increased histamine release to 150% of basal levels (31).

For this purpose, the present placebo-controlled study investigated the impact of modafinil on resting-state brain functional connectivity between distinct cerebellar regions and the rest of the brain in a cohort of 50 participants. We conducted assessments before and after the administration of a single dose of modafinil (n=25) or placebo (n=25). We chose a 100 mg dose to minimize potential side effects, especially considering that we are testing it on an elderly population. However, it should be recognized that

higher doses, ranging from 200-400 mg, may lead to a more pronounced modification of the inhibitory/excitatory balance, as the compound could have a greater impact on glutamatergic and GABAergic neurotransmission (32). The effect of modafinil on cerebellar-neocortical functional connectivity was assessed through a series of seed-based analyses, one for each of the twenty-five cerebellar sub-regions that were defined from the anatomical labeling atlas 3 (AAL-3) (33). In addition, we conducted a network-level analysis to investigate the spatial covariation between cerebellar-specific functional connectivity changes and the density of receptor/transporter subtypes (34), using quantitative information obtained from high-resolution, in vivo, whole-brain PET atlases of major neurotransmitter receptors/transporters (35).

Methods and Materials

Ethical approval

The current study involves re-analyzing data previously published by our group (15, 17), made possible by recent methodological advancements. The novelty of the current research lies in the systematic examination of the effect of modafinil on the connectivity between a distinct subset of cerebellar regions and the neocortex. Moreover, we also provide, for the first time, evidence of a spatial correlation between these physiological effects and the expression of specific receptors/transporters. The current study received approval from the Research and Ethics Committee, and all participants provided written informed consent. All procedures adhered to the ethical standards outlined in the Declaration of Helsinki.

Study Participants

Participants underwent screening to exclude historical of psychiatric, neurological, or medical issues such as hypertension, cardiac disorders, or epilepsy, utilizing the Millon test and a clinical evaluation. General exclusion criteria encompassed visual or motor disabilities, current use of psychoactive medications, or a history of alcohol misuse or psychoactive drug abuse. No subjects had present or past exposure to psychostimulants. None of the study participants were active smokers, and all consumed a typical amount of 1-2 cups of Italian espresso daily. Volunteers were instructed to maintain their usual nicotine and caffeine consumption and to abstain from alcohol for 12 hours before the study began. The study enrolled twenty-six right-handed young males (as determined by the Edinburgh Handedness Inventory), 25-35 years old, with an education level of 13 years. Additionally, the study included twenty-four older adults (aged 57-75 years) with similar educational backgrounds (11±3.9 years). Eligibility of older adults was confirmed through physical and neurological assessments by an experienced neurologist, along with a detailed radiological review to ensure MRI compatibility. The presence of cognitive deterioration was excluded using the Mini-Mental State Examination (MMSE) (36).

Study Protocol

After obtaining consent, participants were randomly assigned in a double-blind manner to receive either a single dose of modafinil (n=25) or a visually indistinguishable placebo (n=25). The following day, participants were queried about any potential side effects, particularly sleep disruptions; all except one reported no changes in sleep patterns or other side effects from modafinil. Following administration, participants underwent two fMRI scans: one before and another three hours after intake, aligning with the drug's pharmacokinetic peak (37). The modafinil and placebo groups were comparable regarding educational levels, age, and cognitive functioning at enrollment (15, 17).

MRI data collection

MR images were acquired with a Philips Achieva 3T Scanner (Philips Medical Systems, Best, The Netherlands) using an eight-channel head coil. High-resolution structural images were collected at the end of the three rs-fMRI runs through a 3D T_1-weighted sequence employing the following parameters: sagittal, matrix 256×256, FOV 256 mm, slice thickness 1 mm, no gaps, in-plane voxel size 1 mm x 1 mm, flip angle 12°, TR=9.7 ms and TE=4 ms. Rs-fMRI BOLD signals were collected in three runs for younger participants and two runs for the elderly, each run lasting four minutes. Rs-fMR images were acquired using T_2*-weighted echo planar imaging (EPI) free induction decay (FID) sequences and applying the following parameters: TE 35 ms, matrix size 64×64, FOV 256 mm, in-plane voxel size 4×4 mm, flip angle 75°, slice thickness 4 mm and no gaps. 140 functional volumes consisting of 30 transaxial slices were acquired per run. The protocol for young subjects consisted of three runs with a TR of 1.67 s, whereas the protocol for older subjects included two runs with a TR of 1.7 s.

MR data analysis

The data were preprocessed and analyzed using the Conn toolbox, version 22 (https://web.conn-toolbox.org, 38). The standard preprocessing pipeline consisted of the following steps: realignment, slice timing correction, outlier detection, segmentation, MNI-space normalization, and smoothing. Functional data were realigned using the SPM realign & unwarp procedure, where all scans were co-registered to a reference image (first scan of the first session) using a least squares approach and a 6-parameter (rigid body) transformation and resampled using b-spline interpolation. The temporal misalignment between different slices of the functional data was corrected following the SPM slice-timing correction (STC) procedure, using "sinc" temporal interpolation to resample each slice BOLD time series to a common mid-acquisition time. Potential outlier scans were identified using ART as acquisitions with framewise displacement above 0.9 mm or global BOLD signal changes above 5 standard deviations (39). Functional and anatomical data were normalized into standard MNI space, segmented into grey matter, white matter, and CSF tissue classes, and resampled to 2 mm isotropic voxels following a direct normalization procedure (40) using SPM unified segmentation and normalization algorithm (41) with the default IXI-549 tissue probability map template. Finally, functional data were smoothed using spatial convolution with a Gaussian kernel of 8 mm full-width half maximum (FWHM).

In addition, functional data were denoised using a standard denoising pipeline, including the regression of potential confounding effects characterized by white matter time-series, CSF time-series, motion parameters and their first order derivatives (12 factors), outlier scans, and linear trends (2 factors) within each functional run, followed by bandpass frequency filtering of the BOLD time series between 0.008 Hz and 0.09 Hz. CompCor (42, 43) noise components within white matter and CSF were estimated by computing the average BOLD signal and the largest principal components orthogonal to the BOLD average, motion parameters, and outlier scans within each subject's eroded segmentation masks. From the number of noise terms included in this denoising strategy, the effective degrees of freedom of the BOLD signal after denoising were estimated to range from 122.4 to 192.3 (average 161.1) across all subjects. Seed-based analysis (https://web.conn-toolbox.org/fmri-methods/connectivity-measures/seed-based) were obtained as the Fisher-transformed bivariate correlation coefficients between each seed and all the other voxels in the brain and was performed for twenty-six cerebellar sub-regions that were defined from (CONN-integrated) Automated anatomical labeling atlas 3 (AAL-3) (33): left and right Crus I and II; left and right Lobules III, IV/V, VI, VIIB, VIII, IX, X of cerebellar hemisphere; Lobules I-II, III, IV/V, VI, VII, VIII, IX, X of the Vermis.

Whole-brain voxel-based mapping

All the rs-fMRI analyses were conducted using a General Linear Model (GLM) methodology. Each voxel underwent a separate GLM estimation, with first-level connectivity measure [expressed as the difference in z-Fisher transformed connectivity strength between post-treatment (T2) and pretreatment (T1) conditions] at that voxel serving as the dependent variable, and the two groups (modafinil and placebo) acting as the independent variable. Voxel-level hypotheses were evaluated utilizing multivariate parametric statistics with random effects across subjects and sample covariance estimation across multiple measurements. Inferences were made at the level of individual clusters (groups of contiguous voxels). Cluster-level inferences were derived from parametric statistics based on Gaussian Random Field theory (44). Results were thresholded using a combination of a cluster-forming $p<0.001$ voxel-level thresholds, and a false discovery rate (FDR) corrected $p<0.05$ cluster-size threshold (45).

Spatial overlaps between the rs-fMRI maps and PET atlas

A correlation analysis examined the spatial overlap between the rs-fMRI maps (obtained from our seed-based analyses described above) and PET-derived maps (35). Using FreeSurfer (https://surfer.nmr.mgh.harvard.edu/), the non-thresholded z-maps generated by CONN processing were projected onto the fsaverage5 surface through a combination of the mri_vol2surf and mri_surf2surf command lines. Subsequently, the mri_segstats command line was employed to extract the mean z-value for each of the 100 components of the Schaefer Atlas (7 networks) (46). The receptor/transporter density within each of the 100 components of the Schaefer Atlas (7 networks) is available at https://github.com/netneurolab/hansen_receptors (34). Based on a previous study on the receptor/transporter-binding profile of modafinil at low to

moderate doses (4), we assessed the serotoninergic (transporter: 5-HTT), dopaminergic (receptors: D_1, D_2; transporter: DAT), noradrenergic (transporter: NET), and histaminergic (receptor: H_3) neurotransmission. The BrainSMASH method (35), implemented in the neuromaps package at https://github.com/netneurolab/neuromaps, was employed to address the issue of spatial autocorrelation in brain maps. This approach generates a population of 1000 null receptor maps that retain the same spatial autocorrelation as the original data (as well as the same mean and variance). Each correlation was then repeated with each null map to obtain a null distribution, which was used to derive a p-value that accounts for spatial autocorrelation.

Results

Whole-brain voxel-based analysis

Whole-brain voxel-wise based analysis revealed that modafinil, compared to placebo, selectively modified the cortical functional connectivity of five cerebellar subregions, including Crus I and multiple areas of the Vermis. This process involved cortical areas of high-order networks, such as the DMN and FPCN, as well as primary sensory areas like the visual cortex. In line with our previous study (17), we observed that the Lobules VII-VIII of the Vermis showed altered functional coupling with the visual cortex. Moreover, as hypothesized, the left Crus I showed increased functional connectivity with the lateral and medial prefrontal regions, which are part of the FPCN and DMN, respectively (**Fig. 1**). Interestingly, Lobules I-II of the Vermis showed increased functional connectivity with both the left and right dorsal anterior cingulate cortex (dACC), which is part of the SVAN (**Fig. 2**). The SVAN is implicated in various processes related to stimulus valence and emotional domains, as well as in alerting and task control (47). While the functional connectivity of the visual cortex was reduced with the Lobule VII (**Fig. 3**), it was increased with the Lobule VIII (**Fig. 4**). Lastly, Lobule IX of the Vermis complex showed increased functional connectivity with the left medial prefrontal cortex, which is part of the DMN (**Fig. 5**). Importantly, we did not find interaction effects between treatment and age group (**Suppl. Table 1**).

Spatial covariance of the receptors/transporters with functional connectivity changes

We examined the presence of a significant spatial correlation between the metrics of functional connectivity and receptor/transporter density. Within the modafinil group, the increased functional connectivity of the left Crus I exhibited a positive association with the density of D_2 receptors in the left hemisphere (R=0.47, SA corrected p=0.016; **Fig. 6A**; **Suppl. Table 2**). Additionally, the enhanced functional connectivity of Lobules I-II of the Vermis complex demonstrated a positive association with the expression of H_3 receptors in the left hemisphere (R=0.43, SA corrected p=0.01; **Fig. 6B**; **Suppl. Table 3**). The functional connectivity reduction of Lobules VII of the Vermis was positively associated with the density of D_2 in the left hemisphere (R=0.39, SA corrected p=0.039; **Fig. 6C**; **Suppl. Table 4**). Lastly, the increased functional connectivity modification of Lobule VIII exhibited negative

associations with H_3 receptors in both left (R=-0.39, SA corrected p=0.044) and right (R=-0.37, SA corrected p=0.027; **Fig. 6D**; **Suppl. Table 5**) hemispheres. No significant spatial overlaps were found between the increased connectivity of Lobules IX of the Vermis and the maps from tested receptors or transporters (**Suppl. Table 6**).

Discussion

Traditionally linked with motor control, the cerebellum is now acknowledged for its broader influence on cognitive functions and their associated cortical networks (18, 19). Based on the results of an exploratory analysis conducted in our previous study (17), here we comprehensively assessed the modulation of cerebellar-cortical functional connectivity induced by modafinil by looking at the contribution of distinct cerebellar regions. The present findings are in line with the evolving understanding of the cerebellum's role over the past three decades.

Modafinil altered the connectivity between distinct cerebellar sub-regions and specific components of large-scale networks. This phenomenon could be explained by the fact that cortical network boundaries are often relative and depend on the threshold used to define them, as seen when comparing different functional atlases (48). The main recognized large-scale networks integrate several subsystems, each characterized by specific anatomical connections and distinct tissue-level receptor and transporter profiles, which could explain the heterogeneous modulation induced by a drug. We found a significant colocalization of the major connectivity changes with the expression of D_2 and H_3 receptors. On the other hand, no spatial overlap was found between the DAT and NET levels and the modafinil-induced cerebellar-cortical connectivity changes. This phenomenon could be explained by the fact that while transporter activity is relevant for modulating neurotransmitter concentrations, receptors are the primary beneficiaries of this increase and the effectors that drive neural changes in a spatial context.

By extending our previous finding of increased connectivity between V1 and the cerebellum obtained with graph theory approaches on the same dataset (17), the present results indicate that modafinil alters the connectivity between Lobules VII-VIII of the Vermis and the visual cortex. This evidence agrees with recent findings by Yeo's group (28), showing that vermis regions are functionally coupled with the neocortical visual cortex. Notably, here we demonstrate that the changes in functional connectivity of Lobule VII/VIII of the Vermis overlap with neocortical regions with low levels of D_2 and H_3 receptors. Therefore, these findings suggest that this phenomenon may not be directly linked to dopaminergic but instead associated with histaminergic modulation.

Perhaps more interestingly, our study revealed that modafinil significantly alters the cerebellar connectivity of both Crus I and posterior regions of the Vermis (Lobule IX) with the lateral and medial regions of the frontal cortex, encompassing respectively the anterior portions of the FPCN and DMN. From a neurophysiological perspective, Crus I has been linked to cerebral association areas within the dorsolateral prefrontal cortex and parietal association cortex, which are critical hubs of the FPCN (20, 26, 27).

Additionally, Krienen and Buckner (49) demonstrated that Crus I functionally couples with the DMN and, in particular, with the medial prefrontal cortex, a region whose activity is essential in promoting the crosstalk and synergic integration of the DMN with the FPCN and other attentional networks (49-53). At the functional level, the Crus is implicated in working memory, classical conditioning, and sequence learning (54), whereas the Vermis is additionally associated with emotional behavior and explicit memory retrieval (54).

Further support for our findings comes from theoretical models demonstrating how the phylogenetic expansion of the neocerebellum, especially in the Crus regions, correlates with an enlargement of cortico-cerebellar connections, predominantly prefrontal, reflecting concurrent evolution in these areas in hominids and humans. Moreover, anatomical and human fMRI studies have shown that lesions in the posterior lobe of the cerebellum, particularly in the Crus, can lead to executive function deficits similar to those observed with prefrontal lesions (55). Thus, increased connectivity between the Crus and the frontal region of the FPCN suggests a potential mechanism by which modafinil influences executive and attentional processing, contributing to its effects on cognitive functions (56). The spatial covariance of receptors/transporters with functional connectivity changes suggests that the increased connectivity of cerebellum Crus I is associated with modafinil's modulation of the dopaminergic system, particularly with the binding of D_2 receptors. Although there is still much to clarify about how modafinil can determine neural modulation through actions on D_2 receptors, the colocalization of the connectivity changes of Crus I with the D_2 receptors agrees with the described involvement of the dopaminergic system in producing the behavioral (57) and wake-promoting effects of modafinil (6). Moreover, our findings align with data from transcortical electrode recordings in awake rats, demonstrating that modafinil modulates the power spectra at frequencies above 4 Hz in the prefrontal cortex (58). Interestingly, the prefrontal cortex was found to be significantly hyper-connected with cerebellar areas involved in executive functions (58) and is rich in dopamine receptors, which, as suggested by evidence from wild-type mice, are essential for modafinil-induced wakefulness (6).

Beyond its connections with the prefrontal cortex, the Vermis exerts a modulatory influence on the subcortical nodes of the salience network. Reports indicate that Vermis stimulation improves certain psychiatric disorders (59) and increases theta activity associated with emotion and memory (60). Thus, the increased connections between the Vermis and regions of the salience network, alongside heightened arousal, align with our previous findings demonstrating modafinil's modulation of the insula and its effect on motivation and salience control of pleasure. Interestingly, we noted an overlap in increased connectivity of cerebellar Vermis I/II with regions rich in H_3 receptors. Histamine H_3 receptors are present in the central nervous system and, to a lesser extent, in the peripheral nervous system, where they function as autoreceptors in presynaptic histaminergic neurons. They regulate histamine turnover by inhibiting feedback on histamine synthesis and release (61). The brain's histaminergic arousal system is negatively regulated by these H_3 autoreceptors, whose removal leads to a sustained increase in histamine turnover. Moreover, this receptor has been proposed as a target for treating sleep disorders (62). Thus, the spatial overlap between increased

Vermis I/II connectivity with neocortical regions of the salience network and H_3 receptors could offer additional evidence of modafinil's role in histaminergic modulation, suggesting potential direct involvement in the arousal system and regulation of sleep-wake cycles.

Notably, we did not observe subcortical variations in cerebellar connectivity with subcortical structures like the striatum and hippocampus, which are characterized by a high density of modafinil-like receptors. We speculate that modafinil may not directly influence the connectivity between the cerebellum and subcortical structures (as striatal-cerebellar connections are primarily related to motor circuitry) but rather modulates cortical areas, such as the orbitofrontal and medial cortices, which are abundant in dopaminergic receptors and serve as direct targets for the connectivity changes in crus I.

The present study has some limitations that need to be acknowledged. First, while the study's primary aim was to understand the neural processes linked to acute modafinil administration, one significant constraint is the absence of an examination of behavioral outcomes following short-term use of modafinil. In this respect, one must be cautious when linking resting-state connectivity to cognitive functions since spontaneous activity does not necessarily inform about the brain's response to cognitive tasks.

Second, the use of an existing database limited our possibility of preregistering the working hypotheses and the statistical analyses, which is a fundamental step towards transparent, rigorous, and replicable research. Nevertheless, we emphasize that the motivation for the current analysis originates from a consideration of recent methodological advancements concerning the parcellation of the cerebellum (33) and the availability of receptor/transporter maps (35). Regarding the first methodological aspect, we note that methods for individualized parcellation are still lacking. The potential biases due to imperfect correspondence with individual anatomy could lead to less precise sampling of the average signal and negatively impact the estimation of functional connectivity. One possible limitation concerning the receptor/transporter maps is the age-related effect on the number of receptors in the neocortex (63). However, for critical receptors/transporters, the age range of the PET database (48.4±16.9 years and 32.5±9.7 years for D_2, and 61±11 years for DAT) closely matched the age range of our groups (46.2±20.3 years for the modafinil subset and 46.0±20.2 years for the placebo group). Conversely, the age difference between maps may be more relevant for H_3 and 5-HT_{2A} receptors, as the populations used for sampling these receptors had younger mean ages of 31.7±9.0 and 22.6±2.7 years, respectively.
In addition, the current dataset lacks physiological data, which limits our ability to exclude potential interference from cardiac and respiratory signals. Moreover, the present voxel resolution (4x4x4 mm^3) restricts accurate analysis of connectivity patterns concerning small structures such as the Ventral Tegmental Area (VTA) and the raphe nuclei, the sources of dopaminergic and serotonergic neurotransmission. These limitations highlight opportunities for future research, especially using higher spatial resolution images (achievable with a 7T scanner) and, at a minimum, subject-by-specific segmentation of mesencephalic nuclei. Lastly, we acknowledge as a limitation of the manuscript that rs-fMRI data were collected in three sessions for younger participants and in two sessions for older participants. This

discrepancy could affect the correlation coefficients, as measurement error tends to decrease with longer scanning periods.

In conclusion, our study provides new insights into the neural mechanisms of modafinil. Specifically, it not only reveals how modafinil influences the pattern of cerebellar connectivity with the neocortex, revealing a specific functional connectivity modulation of the Crus I and multiple areas of the Vermis, but also demonstrates the selective association of these modulations with the spatial distribution of dopaminergic and histaminergic receptors and serotonin transporters.

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Disclosures

The authors report no biomedical financial interests or potential conflicts of interest.

Supplement Description:

Tables S1-S6

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Figure legends

Figure 1. Modafinil-Induced changes in Crus I functional Connectivity. Panel A shows the seed location for the seed-to-voxel functional connectivity analysis, highlighted in green. Panel B shows the surface unthresholded cortical maps illustrating the topographic distribution of modafinil-induced changes in functional connectivity. Panel C shows statistical thresholded maps, with a cluster-forming threshold of $p < 0.001$ at the voxel level and a family-wise corrected p-FDR < 0.05 at the cluster-size level. For panels B and C, yellow-red/light blue-blue clusters indicate increased/decreased functional connectivity, respectively. Panel D reports the violin plots showing the group distribution (red: modafinil, MOD; blue: placebo, PCB) of functional connectivity within the significant clusters. The box shows the quartiles of the dataset while the whiskers indicate the rest of the distribution, represented as multiple (by default, as in this case: 1.5) of the "inter-quartile range" (IQR), which is the range between the lower and upper quartile covered by the inner box. The IQRs function evaluates observations outside this range as potential "outliers", which are then displayed outside the whiskers.

Figure 2. Modafinil-induced changes in Lobule I-II of Vermis functional connectivity. Panel A shows the seed location, which is highlighted in green. Panel B shows the surface unthresholded cortical maps illustrating the topographic distribution of modafinil-induced changes in functional connectivity. Panel C shows statistical thresholded maps, with a cluster-forming threshold of $p < 0.001$ at the voxel level and a family-wise corrected p-FDR < 0.05 at the cluster-size level. For panels B and C, yellow-red/light blue-blue clusters indicate increased/decreased functional connectivity, respectively. Panel D reports the violin plots showing the group distribution (red: modafinil, MOD; blue: placebo, PCB) of functional connectivity within the significant clusters.

Figure 3. Modafinil-Induced changes in Lobule VII of Vermis functional connectivity. Panel A shows the seed location, which is highlighted in green. Panel B shows the surface unthresholded cortical maps illustrating the topographic distribution of modafinil-induced changes in functional connectivity. Panel C shows statistical thresholded maps, with a cluster-forming threshold of $p < 0.001$ at the voxel level and a family-wise corrected p-FDR < 0.05 at the cluster-size level. For panels B and C, yellow-red/light blue-blue clusters indicate increased/decreased functional connectivity, respectively. Panel D reports the violin plots showing the group distribution (red: modafinil, MOD; blue: placebo, PCB) of functional connectivity within the significant clusters.

Figure 4. Modafinil-induced changes in Lobule VIII of Vermis functional connectivity. Panel A shows the seed location, which is highlighted in green. Panel B shows the surface unthresholded cortical maps illustrating the topographic distribution of modafinil-induced changes in functional connectivity. Panel C shows statistical thresholded maps, with a cluster-forming threshold of $p < 0.001$ at the voxel level and a family-wise corrected p-FDR < 0.05 at the cluster-size level. For panels B and C, yellow-red/light

blue-blue clusters indicate increased/decreased functional connectivity, respectively. Panel D reports the violin plots showing the group distribution (red: modafinil, MOD; blue: placebo, PCB) of functional connectivity within the significant clusters.

Figure 5. Modafinil-induced changes in Lobule IX of Vermis functional connectivity. Panel A shows the seed location, which is highlighted in green. Panel B shows the surface unthresholded cortical maps illustrating the topographic distribution of modafinil-induced changes in functional connectivity. Panel C shows statistical thresholded maps, with a cluster-forming threshold of $p < 0.001$ at the voxel level and a family-wise corrected p-FDR < 0.05 at the cluster-size level. For panels B and C, yellow-red/light blue-blue clusters indicate increased/decreased functional connectivity, respectively. Panel D reports the violin plots showing the group distribution (red: modafinil, MOD; blue: placebo, PCB) of functional connectivity within the significant clusters.

Figure 6. Scatterplot showing the relationships between rs-fMRI outcomes and receptor/transporter density across Schaefer components. Panel A displays the relationship between the strength of Crus I functional connectivity changes and the density of the D_2-receptors. Panel B shows the relationship between the strength of functional connectivity changes in Lobules I-II of the Vermis and the density of H_3-receptors. Panel C illustrates the relationship between the strength of functional connectivity changes in Lobules VII of the Vermis and the density of D_2 receptors. Panel D depicts the relationship between the strength of functional connectivity changes in Lobules VIII of the Vermis and the density of H_3 receptors.