Biological Psychiatry: CNNI

Archival Report

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 used to treat narcolepsy but is also used as an off-label cognitive enhancer. Functional magnetic resonance imaging 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 functional magnetic resonance imaging connectivity were estimated in 50 participants from scans acquired pre- and postadministration of a single (100 mg) dose of modafinil (n = 25) or placebo (n = 25). Using specific cerebellar regions as seeds for voxelwise analyses, we examined modafinil's modulation of 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.

CONCLUSIONS: 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.

https://doi.org/10.1016/j.bpsc.2024.11.010

Modafinil 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 because 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 to be somewhat unspecific because modafinil can boost the release of numerous neurotransmitters (3,4). Modafinil directly binds the dopamine transporter (DAT) and norepinephrine transporter (NET), 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), and 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 (gamma-aminobutyric acid) (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 a 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 of a drug given that task-evoked activity is

associated with only a small part (<5%) of total brain energy consumption (10). Alternatively, the study of the spontaneous low-frequency fluctuations in the blood oxygen 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 brain energy consumption, providing insights on how the functional connectome is organized at rest to eventually support task-evoked activities (11). Resting-state fMRI (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 participants increased the functional connection strength across task-positive networks, including the so-called frontoparietal control network (FPCN), dorsal attention network, and salience/ventral attention network (13–15). More recent studies (14) have provided additional support to these findings, showing that modafinil enhanced 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. 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 cortico-cerebellar 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 because 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 rather 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 cerebellar-cortical connectivity is needed to assess its anatomical-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 the DMN and FPCN. Moreover, it is still unclear whether physiological effects, measured through indices of neurovascular coupling, are related to the pharmacological mechanisms that underlie 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 serotoninergic (transporter: 5-HT transporter), 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 anticipated that the expression maps of these transporters and D_1/D_2 receptors might show significant correlations with rs-fMRI maps. Additionally, considering that histamine is a key neurotransmitter in regulating the sleep-wake cycle (29) and one of modafinil's primary targets, we explored the potential involvement of H_3 receptors (30), which are 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, in the current placebo-controlled study, we 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 were testing it on an elderly population. However, it should be recognized that higher doses, ranging from 200 to 400 mg, may lead to a more pronounced modification of the inhibitory/excitatory balance because 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 25 cerebellar subregions that were defined from the Anatomical Labeling Atlas 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 positron emission tomography (PET) atlases of major neurotransmitter receptors/transporters (35).

METHODS AND MATERIALS

Ethical Approval

The current study involved reanalyzing data previously published by our group (15,17), which was 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 histories 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 participants had present or past exposure to psychostimulants. None of the study participants were active smokers, and all consumed a typical amount of 1 to 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 26 right-handed young men (as determined by the Edinburgh Handedness Inventory) who were 25 to 35 years old, with an education level of 13 years. The study also included 24 older adults (aged 57–75 years) with similar educational backgrounds (11 $\pm$ 3.9 years). Eligibility of older adults was confirmed through physical and neurological assessments by an experienced neurologist together with a detailed radiological review to ensure MRI compatibility. The presence of cognitive deterioration was excluded using the Mini-Mental State Examination (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 2 fMRI scans, one before and another 3 hours after intake, consistent with the drug's pharmacokinetic peak (37). The modafinil and placebo groups were comparable in terms of 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) using an 8-channel head coil. High-resolution structural images were collected at the end of the 3 rs-fMRI runs through a 3-dimensional T1-weighted sequence using the following parameters: sagittal, matrix 256 $\times$ 256, FOV 256 mm, slice thickness 1 mm, no gaps, in-plane voxel size 1 $\times$ 1 mm, flip angle 12°, TR 9.7 ms, and TE 4 ms. rs-fMRI BOLD signals were collected in 3 runs for younger participants and 2 runs for the elderly, each run lasting 4 minutes. rs-fMR images were acquired using T2*-weighted echo planar imaging free induction decay sequences and applying the following parameters: TE 35 ms, matrix size 64 $\times$ 64, FOV 256 mm, in-plane voxel size 4 $\times$ 4 mm, flip angle 75°, slice thickness 4 mm, and no gaps. One hundred forty functional volumes consisting of 30 transaxial slices were acquired per run. The protocol for young participants consisted of 3 runs with a TR of 1.67 seconds, whereas the protocol for older participants included 2 runs with a TR of 1.7 seconds.

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, Montreal Neurological Institute (MNI) space normalization, and smoothing. Functional data were realigned using the SPM realign & unwarp procedure, where all scans were coregistered to a reference image (the 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 procedure, using "sinc" temporal interpolation to resample each slice BOLD time series to a common midacquisition time. Potential outlier scans were identified using Artifact Detection Tools (ART) as acquisitions with framewise displacement above 0.9 mm or global BOLD signal changes above 5 SDs (39). Functional and anatomical data were normalized into standard MNI space, segmented into gray matter, white matter, and cerebrospinal fluid tissue classes, and resampled to 2-mm isotropic voxels following a direct normalization procedure (40) using the 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 at half maximum.

In addition, functional data were denoised using a standard denoising pipeline, including the regression of potential confounding effects characterized by white matter time series, cerebrospinal fluid 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 cerebrospinal fluid 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 participant'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 participants. Seed-based analyses (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 were performed for 26 cerebellar subregions that were defined from (CONN-integrated) Automated Anatomical Labeling Atlas 3 (33): left and right crus I and II; left and right lobules III, IV/V, VI, VIIB, VIII, IX, and X of cerebellar hemisphere; lobules I to II, III, IV/V, VI, VII, VIII, IX, and X of the vermis.

Whole-Brain Voxel-Based Mapping

All the rs-fMRI analyses were conducted using a general linear model methodology. Each voxel underwent a separate general linear model estimation, with the first-level connectivity measure (expressed as the difference in Fisher's z-transformed connectivity strength between posttreatment and pretreatment conditions) at that voxel serving as the dependent variable and the 2 groups (modafinil and placebo) acting as the independent variable. Voxel-level hypotheses were evaluated utilizing

multivariate parametric statistics with random effects across participants 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 $p < .001$ voxel-level thresholds and a false discovery rate–corrected $p < .05$ cluster-size threshold (45).

Spatial Overlap 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 nonthresholded *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 used 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 of the receptor/transporter-binding profile of modafinil at low to moderate doses (4), we assessed the serotoninergic (transporter: 5-HT transporter), 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 used 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 voxelwise-based analysis revealed that compared with placebo, modafinil selectively modified the cortical functional connectivity of 5 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. Consistent with our previous study (17), we observed that 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 (Figure 1). Interestingly, lobules I/II of the vermis showed increased functional connectivity with both the left and right dorsal anterior cingulate cortex, which is part of the salience/ventral attention network (Figure 2). The salience/ventral attention network is implicated in various processes related to stimulus valence and emotional domains, as well as in

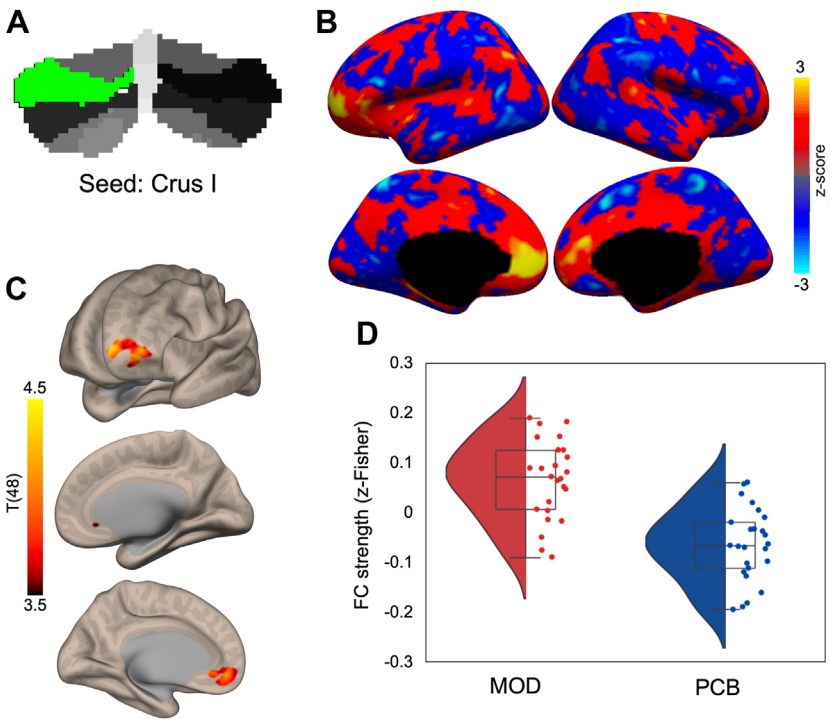

Figure 1. Modafinil-induced changes in crus I functional connectivity (FC). Panel **(A)** shows the seed location for the seed-to-voxel FC analysis, highlighted in green. Panel **(B)** shows the surface unthresholded cortical maps illustrating the topographical distribution of modafinil-induced changes in FC. Panel **(C)** shows statistical thresholded maps, with a cluster-forming threshold of $p < .001$ at the voxel level and a familywise-corrected false discovery rate–corrected $p < .05$ at the cluster-size level. For panels **(B)** and **(C)**, yellow-red/light blue-blue clusters indicate increased/decreased FC, respectively. Panel **(D)** depicts the violin plots showing the group distribution (red: modafinil [MOD], blue: placebo [PCB]) of FC 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 interquartile 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.

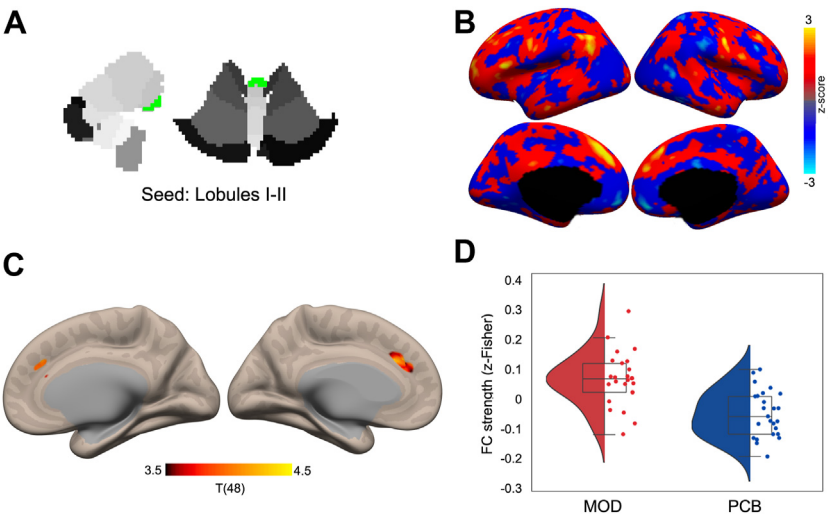

Figure 2. Modafinil-induced changes in lobules I/II of vermis functional connectivity (FC). Panel **(A)** shows the seed location, which is highlighted in green. Panel **(B)** shows the surface unthresholded cortical maps illustrating the topographical distribution of modafinil-induced changes in FC. Panel **(C)** shows statistical thresholded maps, with a cluster-forming threshold of $p < .001$ at the voxel level and a familywise-corrected false discovery rate–corrected $p < .05$ at the cluster-size level. For panels **(B)** and **(C)**, yellow-red/light blue-blue clusters indicate increased/decreased FC, respectively. Panel **(D)** depicts the violin plots showing the group distribution (red: modafinil [MOD], blue: placebo [PCB]) of FC within the significant clusters.

alerting and task control (47). While the functional connectivity of the visual cortex was reduced with lobule VII (Figure 3), it was increased with lobule VIII (Figure 4). Lastly, lobule IX of the vermis complex showed increased functional connectivity with the left medial prefrontal cortex, which is part of the DMN (Figure 5). Importantly, we did not find interaction effects between treatment and age group (Table S1).

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 ($R = 0.47$, spatial autocorrelation–corrected $p = .016$) (Figure 6A and Table S2). 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 $p = .01$) (Figure 6B and Table S3). The functional connectivity reduction of lobule VII of the vermis was positively associated with the density of D_2 receptors in the left hemisphere ($R = 0.39$, SA-corrected $p = .039$) (Figure 6C and Table S4). Lastly, the increased functional connectivity modification of lobule VIII exhibited negative associations with H_3 receptors in both left ($R = -0.39$, SA-corrected $p = .044$) and right ($R = -0.37$, SA-corrected $p = .027$) (Figure 6D and Table S5) hemispheres. No significant spatial overlaps were found between the increased connectivity of lobule IX of the vermis and the maps from tested receptors or transporters (Table S6).

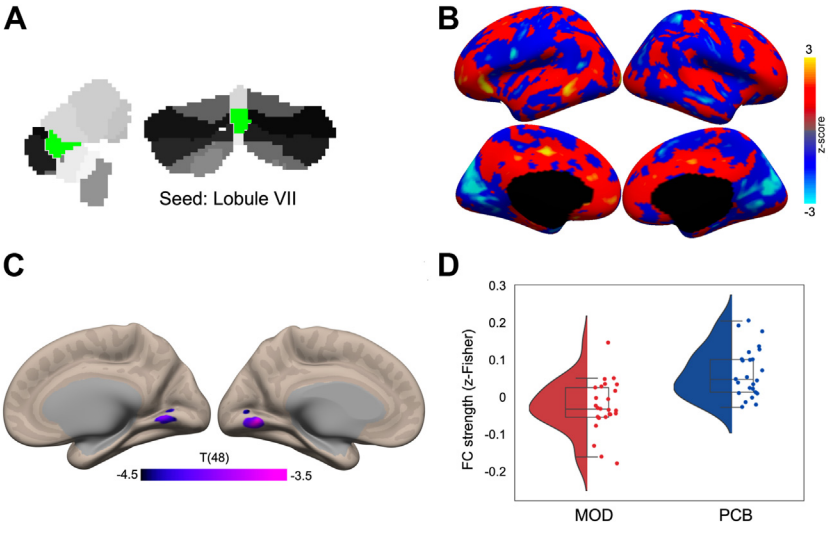

Figure 3. Modafinil-induced changes in lobule VII of vermis functional connectivity (FC). Panel **(A)** shows the seed location, which is highlighted in green. Panel **(B)** shows the surface unthresholded cortical maps illustrating the topographical distribution of modafinil-induced changes in FC. Panel **(C)** shows statistical thresholded maps, with a cluster-forming threshold of $p < .001$ at the voxel level and a familywise-corrected false discovery rate–corrected $p < .05$ at the cluster-size level. For panels **(B)** and **(C)**, yellow-red/light blue-blue clusters indicate increased/decreased FC, respectively. Panel **(D)** depicts the violin plots showing the group distribution (red: modafinil [MOD], blue: placebo [PCB]) of FC within the significant clusters.

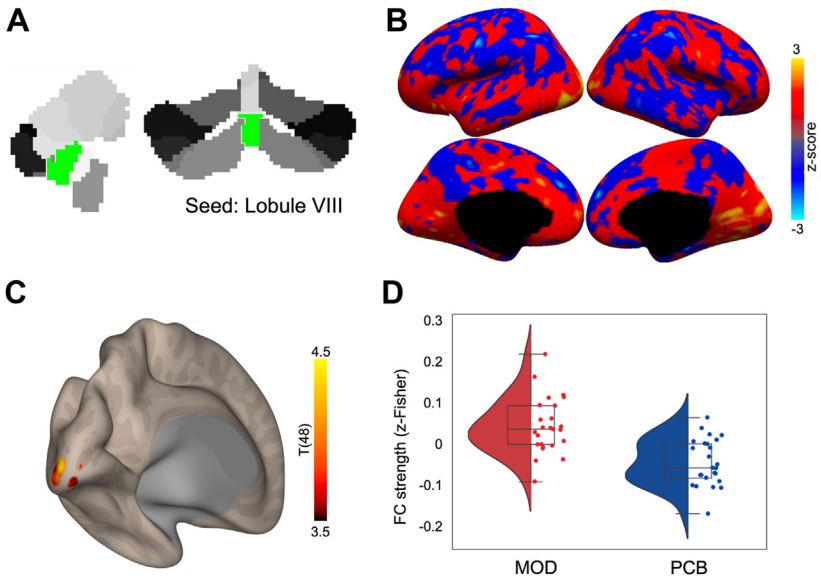

Figure 4. Modafinil-induced changes in lobule VIII of vermis functional connectivity (FC). Panel **(A)** shows the seed location, which is highlighted in green. Panel **(B)** shows the surface unthresholded cortical maps illustrating the topographical distribution of modafinil-induced changes in FC. Panel **(C)** shows statistical thresholded maps, with a cluster-forming threshold of $p < .001$ at the voxel level and a familywise-corrected false discovery rate–corrected $p < .05$ at the cluster-size level. For panels **(B)** and **(C)**, yellow-red/light blue-blue clusters indicate increased/decreased FC, respectively. Panel **(D)** depicts the violin plots showing the group distribution (red: modafinil [MOD], blue: placebo [PCB]) of FC within the significant clusters.

DISCUSSION

The cerebellum, which has traditionally been linked with motor control, 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 current findings are consistent with the evolving understanding of the cerebellum's role over the past 3 decades.

Modafinil altered the connectivity between distinct cerebellar subregions 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 is 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

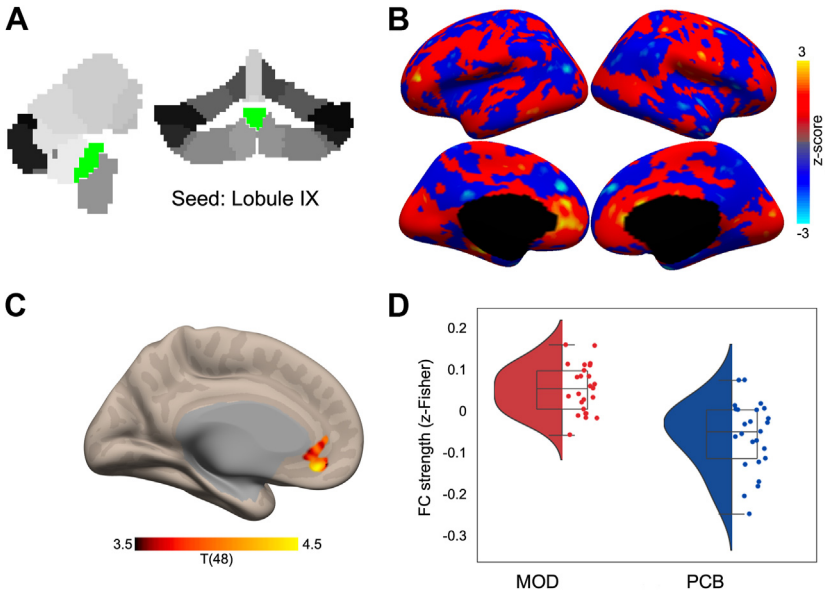

Figure 5. Modafinil-induced changes in lobule IX of vermis functional connectivity (FC). Panel **(A)** shows the seed location, which is highlighted in green. Panel **(B)** shows the surface unthresholded cortical maps illustrating the topographical distribution of modafinil-induced changes in FC. Panel **(C)** shows statistical thresholded maps, with a cluster-forming threshold of $p < .001$ at the voxel level and a familywise-corrected false discovery rate–corrected $p < .05$ at the cluster-size level. For panels **(B)** and **(C)**, yellow-red/light blue-blue clusters indicate increased/decreased FC, respectively. Panel **(D)** depicts the violin plots showing the group distribution (red: modafinil [MOD], blue: placebo [PCB]) of FC within the significant clusters.

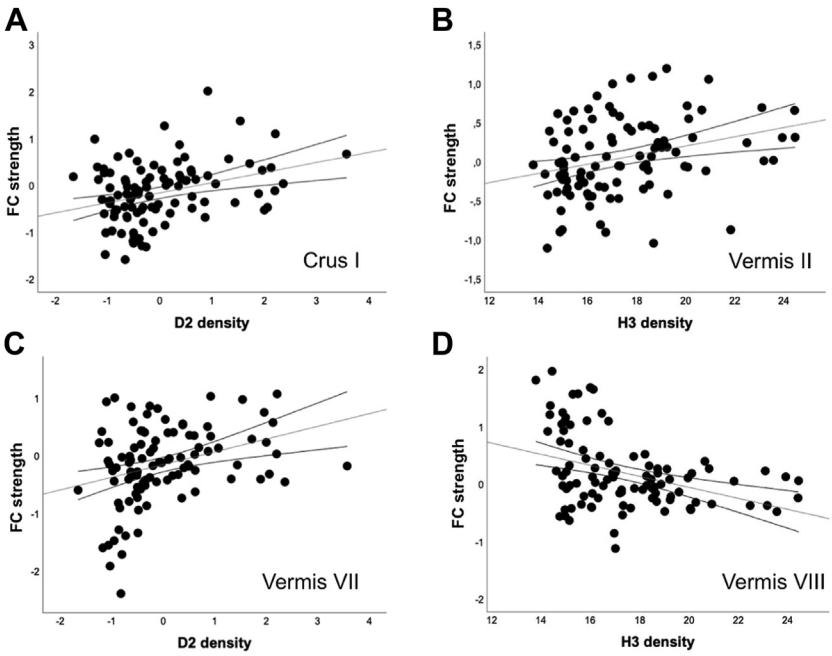

Figure 6. Scatterplot showing the relationships between resting-state functional magnetic resonance imaging outcomes and receptor/transporter density across Schaefer components. Panel **(A)** displays the relationship between the strength of crus I functional connectivity (FC) changes and the density of D_2 receptors. Panel **(B)** shows the relationship between the strength of FC changes in lobules I/II of the vermis and the density of H_3 receptors. Panel **(C)** illustrates the relationship between the strength of FC changes in lobule VII of the vermis and the density of D_2 receptors. Panel **(D)** depicts the relationship between the strength of FC changes in lobule VIII of the vermis and the density of H_3 receptors.

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 current results indicate that modafinil alters the connectivity between lobules VII/VIII of the vermis and the visual cortex. This evidence is consistent with recent findings by Yeo's group (28), showing that vermis regions are functionally coupled with the neocortical visual cortex. Notably, we demonstrated here 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 modulation but may be associated with histaminergic modulation instead.

Perhaps more interestingly, our study revealed that modafinil significantly altered 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 the anterior portions of the FPCN and DMN, respectively. 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 has been implicated in working memory, classical conditioning, and sequence learning (54), whereas the vermis has also been associated with emotional behavior and explicit memory retrieval (54).

Additional 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, that reflect 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, thereby 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 D_2 receptors is consistent with the described involvement of the dopaminergic system in producing the behavioral (57) and wake-promoting effects of modafinil (6). Moreover, our findings are consistent 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 has been found to be significantly

hyperconnected 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, together with heightened arousal, are consistent with our previous findings demonstrating modafinil's modulation of the insula and its effect on motivation and salience control of pleasure. Interestingly, we noted 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, the removal of which 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 (because 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 current 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, it is important to be cautious when linking resting-state connectivity to cognitive functions because spontaneous activity is not necessarily informative 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 toward transparent, rigorous, and replicable research. Nevertheless, we emphasize that the motivation for the current analysis originated from 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 $\pm$ 16.9 years and 32.5 $\pm$ 9.7 years for D_2 and 61 $\pm$ 11 years for DAT) closely matched the age range of our groups (46.2 $\pm$ 20.3 years for the modafinil subset and 46.0 $\pm$ 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 because the populations used for sampling these receptors had younger mean ages of 31.7 $\pm$ 9.0 and 22.6 $\pm$ 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 current voxel resolution ($4 \times 4 \times 4$ mm^3) restricts accurate analysis of connectivity patterns for small structures such as the ventral tegmental area 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, participant-by-specific segmentation of mesencephalic nuclei. Lastly, we acknowledge as a limitation of the study that rs-fMRI data were collected in 3 sessions for younger participants and in 2 sessions for older participants. This discrepancy might have affected the correlation coefficients because measurement error tends to decrease with longer scanning periods.

Conclusions

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.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by Search for Excellence (University "G. d'Annunzio" of Chieti-Pescara [to SDPi]); the Italian Ministry of Health, the AIRAlzh Onlus (to SLS), the Alzheimer's Association—Part the Cloud: Translational Research Funding for Alzheimer's Disease (Grant No. 18PTC-19-602325 [to SLS]), and the Alzheimer's Association—GAAIN (Global Alzheimer's Association Interactive Network) Exploration to Evaluate Novel Alzheimer's Queries (Grant No. GEENA-Q-19-596282 [to SLS]). This work was supported by "Inflammation Profiling in the onset and progression of Parkinson's disease"—Project code: PNRR-MAD-2022-12375706—CUP D73C22002140007 the Italian Ministry of Health—PNRR prot. n. 1436 del 15-04-2022, Financial programm: Piano Nazionale di Ripresa e Resilienza—Missione M6—Componente C2—Investimento 2.1 Valorizzazione e potenziamento della ricerca Biomedica del SSN finanziato dall'Unione europea—NextGenerationEU. – Call "Malattie croniche non trasmissibili, ad alto impatto sui sistemi sanitari e socio-assistenziali" (to SLS). AF and SL acknowledge financial support from Strengthening of research structures and creation of R&D "Innovation Ecosystems," National Recovery and Resilience Plan, mission 4, component 2 investment 1.5, funded by the European Union–NextGenerationEU—VITALITY, ECS00000041 (Grant No. D73C22000840006).

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

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Received Jul 19, 2024; revised Nov 8, 2024; accepted Nov 12, 2024.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.bpsc.2024.11.010.

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