Modafinil Shifts Human Locus Coeruleus to Low-Tonic, High-Phasic Activity During Functional MRI

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Models of cognitive control posit a key modulatory role for the pontine locus coeruleus–norepinephrine (LC-NE) system. In nonhuman primates, phasic LC-NE activity confers adaptive adjustments in cortical gain in task-relevant brain networks, and in performance, on a trial-by-trial basis. This model has remained untested in humans. We used the pharmacological agent modafinil to promote low-tonic/high-phasic LC-NE activity in healthy humans performing a cognitive control task during event-related functional magnetic resonance imaging (fMRI). Modafanil administration was associated with decreased task-independent, tonic LC activity, increased task-related LC and prefrontal cortex (PFC) activity, and enhanced LC-PFC functional connectivity. These results confirm in humans the role of the LC-NE system in PFC function and cognitive control and suggest a mechanism for therapeutic action of procognitive noradrenergic agents.

Models of prefrontal cortex (PFC) function posit a key modulatory role for the ascending brainstem locus coeruleus-norepinephrine (LC-NE) system (*1*–*5*). These models derive largely from studies of non-human primates, rodents, and computational modeling. LC-NE influence confers the PFC capacity to flexibly regulate goal representations by a "gating" mechanism, by modulating the influence of afferent input to PFC ensembles. This enhances the signal-to-noise ratio in active, task-related cortical ensembles. The phasic mode of LC activity drives these adaptive adjustments in gain that facilitate the dynamic reorganization of target networks (*4*) and serve to optimize performance (*3*) in response to the value of current environmental cues or the ongoing assessment of task-related utility. These adjustments are observable on a trial-to-trial basis during cognitive performance.

In vivo and in vitro studies show that phasic LC activity is promoted by decreases in baseline activity (*6*). In this setting, task-related excitatory LC activity is relatively preserved (*7*). NE transporter (NET) inhibitors elevate NE at LC-NE cell bodies, leading to decreased baseline LC activity, and this NET inhibitor effect also facilitates electrotonic coupling and synchronous activity between LC cells (*8*), which can resonate with PFC neurons (*9*).

Most of the fundamental predictions arising from these models have not been tested in humans. It remains unknown how trial-to-trial variation in LC activity relates to task-related PFC activity during human cognition. We addressed this issue by manipulating synaptic NE levels in healthy subjects with modafinil during event-related functional magnetic resonance imaging (fMRI). Modafinil is a nonamphetamine psychostimulant that inhibits the NET and dopamine transporter (DAT) (*10*), elevating synaptic NE and DA levels in the PFC and elsewhere (*11*). Many of modafinil's cognitive and behavioral effects are mediated by adrenergic receptors (*12*). Modafinil also augments LC-mediated pupillary dilation, consistent with enhanced LC phasic responses to task-relevant events (*13*), indicating the potential to optimize cognitive performance through LC-NE modulation. Modafinil improves PFC-dependent cognition in rodents (*14*), healthy humans (*15*, *16*), and patients with attention-deficit/hyperactivity disorder (*17*) and schizophrenia (*18*).

We reasoned that with systemic administration, NET inhibition by modafinil should increase NE levels at LC cell-body autoreceptors, leading to decreased baseline excitatory drive to LC cells (*19*), manifest as a task-independent pontine deactivation. This has been observed with clonidine infusion during resting-state positron emission tomography (PET) (*20*). This effect should in turn promote phasic LC activity (*3*), manifest as increased task-related activity in the LC. In addition, concurrent NET inhibition at terminal fields in the PFC should augment resulting synaptic NE. These effects together should enhance the coupling of LC-NE system neurotransmission with PFC activity, increasing the strength of the negative correlation of tonic LC activity with PFC activation, measured on a trial-to-trial basis by event-related fMRI.

Twenty-one adults (age 33.3 ± 8 years; 12 male) underwent fMRI on 2 days, with mean test-day interval 5.7 ± 4.1 days, after ingesting either modafinil 200 mg or placebo [see supporting online material (SOM) for details]. During fMRI, subjects performed the Preparing to Overcome Prepotency (POP) Task (*21*). Data analyses focused on the effects of modafinil on (i) cognitive control task performance; (ii) tonic, task-independent LC activity; (iii) task-related activity in LC and the cognitive control network; and (iv) connectivity between the LC and cortical cognitive control regions (see SOM).

No significant effects of modafinil were observed on vital signs or subjective state (see SOM). There were small, nonsignificant effects of drug versus placebo on improved accuracy cost (4.1 ± 7.3% versus 6.5 ± 8.5%; $t = -1.08$, $P = 0.14$ in one-tailed paired t test; Cohen's $d = 0.30$) and reaction time (RT) cost (71 ± 65 msec versus 79 ± 62 msec; $t = -0.56$; $P = 0.29$; Cohen's $d = 0.13$) in the full sample. A substantial percentage of the sample performed near ceiling on placebo, making drug effects more difficult to discern. A subgroup with subceiling performance (accuracy ≤95% on placebo high-control cue trials; $n = 11$) exhibited a significantly improved accuracy cost on drug versus placebo (5.4 ± 9.4% versus 12.2 ± 8.1%; $t = 1.964$, $P = 0.039$ in one-tailed paired t test; Cohen's $d = 0.78$).

Compared to placebo, modafinil treatment was associated with significant tonic, task-independent LC deactivation (Fig. 1 and table S1). Task-related drug effects were observed in the LC (Fig. 2A and table S1) and cognitive control areas (Fig. 2B and table S1) (*22*). In the trial-by-trial analysis of LC-PFC connectivity, the drug increased functional connectivity in a task-independent manner in the cognitive control network (Fig. 3 and table S2).

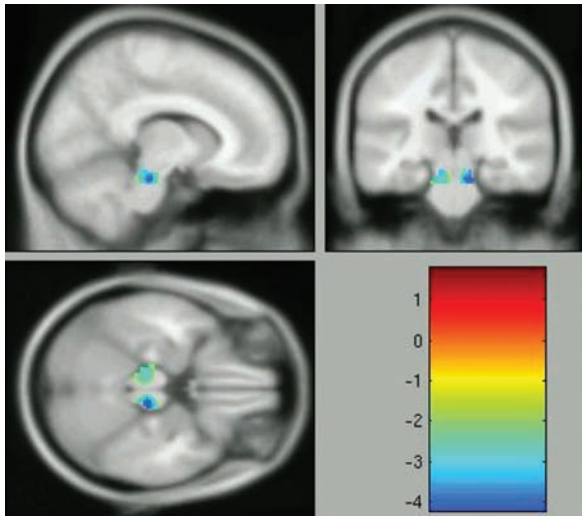

Fig. 1. Statistical parametric maps depict color-coded *t* statistics at each voxel in the brain, for linear contrast evaluating task-independent effect of modafinil on parameter estimates derived from general linear model (GLM) analysis of BOLD signal change. The bilateral pontine clusters, representing LC deactivation as a task-independent drug effect, are statistically significant at $P < 0.05$, corrected for FDR (see table S1 and other SOM). Maps are overlaid on the MNI T1-152 template brain images and shown in midsaggital, coronal, and axial planes, clockwise from top left.

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We also evaluated the relationship of performance to modafinil-induced LC deactivation. Subjects with more negative betas by median split (i.e., stronger LC deactivation on drug; n = 10) exhibited a significantly attenuated drug-related RT cost compared to subjects with weaker LC deactivation on drug (n = 11): 36 ± 34 msec versus 104 ± 71 msec (t = 2.748, P = 0.007 in one-tailed independent samples t test; Cohen's d = 1.30). Across subjects with subceiling placebo-day performance (n = 11, defined above in performance results), there was a significant nonlinear relationship of drug-induced LC deactivation with drug-related RT cost (Fig. 4). In these subjects, stronger LC deactivation was strongly associated with attenuated RT cost.

Modafinil administration was associated with task-independent tonic LC deactivation. Inferences regarding the neurochemical basis of this effect are limited with fMRI. However, this pattern of blood oxygen level–dependent (BOLD) signal change is most consistent with the effects of NET inhibition rather than one of several other modafinil effects: activation of orexinergic neurons, elevated extracellular glutamate and serotonin, and decreased extracellular γ-aminobutyric acid (*12*). In each case, the resulting action on LC-NE cells is excitatory, which would be manifest as LC activation rather than deactivation. In addition, the lack of task-independent drug effects at midbrain dopaminergic nuclei, the ventral tegmental area and substantia nigra, suggests that the task-independent drug effect in the pons is noradrenergic rather than dopaminergic. Studies using more selective NET inhibitors (e.g., atomoxetine) will further clarify the neurochemical mechanisms underlying the LC modulation observed here. Measurement of circulating drug levels would also be useful by allowing correlations with neural and cognitive variables.

It is most likely that our pontine activity changes represent actions at LC neurons, although the activation clusters extend outside the LC proper. This is supported by the following: (i) the activation clusters qualitatively extend throughout the three-dimensional (3D) extent of the LC bilaterally, according to human brain atlases (*23*, *24*); (ii) LC-NE dendrites, which are the likely site of membrane potential changes associated with BOLD signal change (*25*), extend in the pons considerably beyond the LC proper in mammals (*26*, *27*), including humans (*28*); (iii) the activation clusters observed here compare favorably, both qualitatively (3D extent) and quantitatively (location of maxima, cluster size), with those reported in several other studies (*20*, *29*–*32*); (iv) although there are other pontine

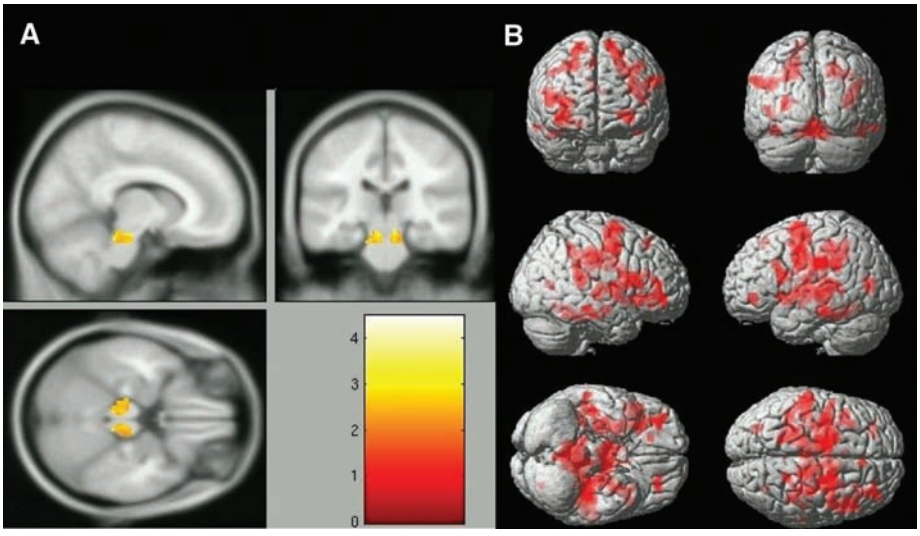

Fig. 2. Statistical parametric maps depict color-coded t statistics at each voxel in the brain, for linear contrast evaluating task-related effect of modafinil on parameter estimates derived from GLM analysis of BOLD signal change. The bilateral pontine clusters in (**A**), representing increased LC activation as a task-related drug effect, are statistically significant at $P < 0.05$, corrected for FDR. The template and spatial coordinates in which the LC clusters are shown are identical to those for the task-independent drug effect shown in Fig. 1. The clusters in the cortex depicted in (**B**) are shown at $P < 0.05$, and the majority are significant at $P < 0.05$ corrected for FDR (table S1).

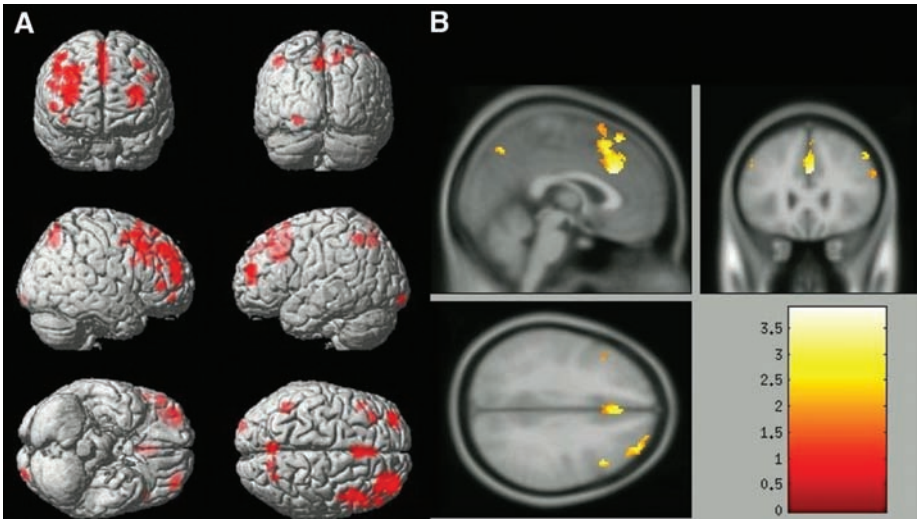

Fig. 3. Statistical parametric maps depicting color-coded t statistics at each voxel in the brain, for linear contrast evaluating task-independent effect of modafinil on the correlation, between the LC seed and all other brain voxels, of trial-by-trial parameter estimates derived from GLM analysis of BOLD signal change. The bilateral pontine clusters observed in Fig. 1, representing significant task-independent LC deactivation, served as the seed in the beta series correlation method. The clusters in the cortex depicted in (**A**) are shown at $P < 0.05$, and the majority are significant at $P < 0.05$ corrected for FDR, as is the large midline anterior cingulate cortex cluster depicted in (**B**) (see table S2 and SOM).

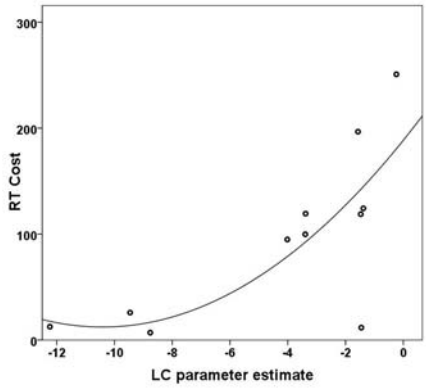

Fig. 4. LC deactivation on modafinil is strongly related to RT cost, across subjects who performed below ceiling (i.e., <95% accuracy) in the high-control (red-cue) condition on placebo (n = 11). For each subject, RT cost is plotted as a function of the mean parameter estimate in the bilateral LC cluster with significant task-independent deactivation on modafinil (see SOM). Quadratic function: R^2 = .56, F = 5.10, df = 2, 8; β (constant) = 188.5, β_1 = 33.8, β_2 = 1.6; P = 0.037.

nuclei containing NE neurons, these do not have ascending projections to the frontal cortex and largely serve autonomic functions. Although the present need for concurrent coverage of the LC and PFC precludes the use of high-resolution fMRI, this method may enhance the localization of pontine nuclei in future studies.

Despite tonic LC deactivation, there was enhancement of task-related activation in the PFC and other terminal fields (*33*). Although this may appear paradoxical, our working model suggests that enhanced task-related phasic LC activity occurs with decreased tonic LC-NE activity. This should be observed during fMRI as a task-independent deactivation, combined with a task-related effect in the opposite direction, which is what we observed. It is possible that the observed PFC effects may be partly mediated by striatal dopamine interactions (*34*), and, because both NET and DAT perform reuptake of extracellular DA in the PFC (*35*), combined NET/DAT inhibition may also increase PFC activity through elevated DA.

Modafinil also increased trial-by-trial LC-PFC coupling. This coupling was negative, consistent with the predicted coincidence of decreased tonic LC activity with positive cortical activation. These results are consistent with evidence that modafinil promotes increased LC-PFC resonance. Because the drug modulated task-independent correlations, tonic LC activity may be the more direct determinant of LC-PFC coupling.

Finally, decreased tonic LC activity in response to modafinil was associated with improved task performance. This relationship is nonlinear, suggesting a particularly sensitive range, at low levels of LC deactivation, where small increments are associated with relatively large adjustments in control. The future use of tasks where subjects perform off-ceiling will facilitate the evaluation of this relationship.

The present results suggest that modafinil shifts the function of the LC-NE system toward a low-tonic/high-phasic pattern of activity to optimize task performance.

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33. These findings do not appear to represent diffuse effects of a drug-induced change in arousal. First, because tonic LC activity is positively related to arousal, the task-independent decrease in LC activity would be consistent with a decreased arousal, rather than an increase. Second, there were no task-independent cortical activity increases on drug. Third, the Profile of Mood States subscales are generally arousal-dependent, and modafinil effects on these measures were minimal. These findings are consistent with an earlier neuroimaging study of modafinil, which found no significant difference between pretreatment and posttreatment activation levels in visual or auditory cortex in response to sensory stimulation (*36*).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/322/5908/1700/DC1
Materials and Methods
SOM Text
Fig. S1
Tables S1 and S2
References

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A Null Mutation in Human *APOC3* Confers a Favorable Plasma Lipid Profile and Apparent Cardioprotection

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Apolipoprotein C-III (apoC-III) inhibits triglyceride hydrolysis and has been implicated in coronary artery disease. Through a genome-wide association study, we have found that about 5% of the Lancaster Amish are heterozygous carriers of a null mutation (R19X) in the gene encoding apoC-III (*APOC3*) and, as a result, express half the amount of apoC-III present in noncarriers. Mutation carriers compared with noncarriers had lower fasting and postprandial serum triglycerides, higher levels of HDL-cholesterol and lower levels of LDL-cholesterol. Subclinical atherosclerosis, as measured by coronary artery calcification, was less common in carriers than noncarriers, which suggests that lifelong deficiency of apoC-III has a cardioprotective effect.

Elevated plasma levels of low density lipoprotein cholesterol (LDL-C) and triglycerides (TGs) are important contributors to premature coronary heart disease (CHD) (*1*–*3*), and genetic variants causing low LDL-C are associated with reduced risk of CHD (*4*). Recently, nonfasting TG was found to be an independent CHD risk factor (*5*, *6*), showing higher predictive power in one study than fasting TG (FTG), the traditional measure, likely because of the atherogenic remnant lipoproteins generated during absorption and clearance of dietary fat (*5*).

To identify genetic factors contributing to FTG and the postprandial TG (ppTG) dietary response, we performed a single high-fat feeding intervention and genome-wide association study (GWAS) in 809 Old Order Amish individuals as part of the Heredity and Phenotype Intervention (HAPI) Heart Study (*7*). Characteristics of these participants are shown in table S1. These individuals were fed a milkshake containing 782 kcal/m^2 body surface area, with 77.6% of these calories from fat, and had blood drawn for lipid levels 0, 1, 2, 3, 4, and 6 hours after the intervention.