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Neural correlates of sleepiness induced by catecholamine depletion

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Abstract

Although extensive indirect evidence exists to suggest that the central dopaminergic system plays a significant role in the modulation of arousal, the functional effect of the dopaminergic influence on the regulation of the sleep-wake cycle remains unclear. Thirteen healthy volunteers and 15 unmedicated subjects with a history of major depressive disorder underwent catecholamine depletion (CD) using oral alpha-methyl-para-tyrosine in a randomized, placebo-controlled, double-blind, crossover study. The main outcome measures in both sessions were sleepiness (Stanford-Sleepiness-Scale), cerebral glucose metabolism (positron emission tomography), and serum prolactin concentration. CD consistently induced clinically relevant sleepiness in both groups ($p < 0.0001$). The CD-induced prolactin increase correlated with CD-induced sleepiness ($r = 0.71$, $p < 0.0001$) but not with CD-induced mood and anxiety symptoms ($p = 0.2$). CD-induced sleepiness correlated with CD-induced increases in metabolism in the medial and orbital frontal cortex, bilateral superior temporal cortex, left insula, cingulate motor area and in the vicinity of the periaqueductal gray. This study suggests that the association between dopamine depletion and sleepiness is independent of the brain reward system and the risk for depression. The visceromotor system, the cingulate motor area, the periaqueductal gray and the caudal hypothalamus may mediate the impact of the dopaminergic system on regulation of wakefulness and sleep.

Keywords

dopamine; norepinephrine; mesencephalon; sleep; hypothalamus; prolactin

1. Introduction

Excessive daytime sleepiness (EDS) is of high clinical and public health importance. Excessive sleepiness has been implicated in approximately 16% of motor vehicle accidents, up to 50% of work-related accidents and up to 25% of home-based accidents (Horne and

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Reyner, 1995; Leger, 1994). EDS has also been associated with psychiatric and medical conditions (Hasler et al., 2005). However, despite its high importance, the neurobiology of EDS is not well understood.

A variety of indirect evidence suggests that the central dopaminergic system plays a major role in sleep-wake regulation. The wake-promoting effect of amphetamine-like stimulants and modafinil has been associated with modulation of the dopaminergic system (Boutrel and Koob, 2004; Qu et al., 2008), and sleepiness in subjects with Parkinson's Disease has been associated with dysfunction of the dopaminergic system (Rye and Jankovic, 2002). Additionally, common sleep pathways in simple organisms such as *Drosophila* have involved dopamine (Andreic et al., 2005). Despite this indirect evidence, it has been difficult to substantiate the mechanistic role of dopamine in sleep-wake regulation in higher mammals. Dopaminergic cells in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) do not change their mean firing rate across the sleep-wake cycle (Trulson et al., 1981), and it has been proposed that these dopaminergic neurons play a role in the maintenance of behavioral arousal but not in the sleep-wake regulation itself (Jones et al., 1973). Recently, however, wake-active dopaminergic neurons have been identified in the ventral periaqueductal gray matter (Lu et al., 2006a) and in the caudal hypothalamus (Leger et al.) where dopamine cells are known to regulate prolactin levels (Moore, 1987). These cells have extensive reciprocal connections with sleep-wake regulation and endocrine systems and thus may support an ascending dopaminergic waking influence (McCarley, 2007).

An instructive paradigm for investigating the relationship between catecholaminergic function and sleepiness more directly in humans involves the behavioral and neural response to catecholamine depletion (CD), achieved by administering alpha-methyl-paratyrosine (AMPT; (Berman et al., 1999; Hasler et al., 2004), a competitive inhibitor of the rate-limiting enzyme in catecholamine synthesis, tyrosine hydroxylase (Nagatsu et al., 1964). AMPT-administration decreases catecholamine transmission by depleting central DA and NE stores, as evidenced by reduced concentrations of catecholamines and their metabolites in plasma, urine and cerebrospinal fluid (Mignot and Laude, 1985; Stine et al., 1997), and by directly measuring the effects of AMPT with dopaminergic imaging agents (Abi-Dargham et al., 2000; Laruelle et al., 1997; Martinez et al., 2009; Riccardi et al., 2008; Verhoeff et al., 2003). Previous studies on catecholamine depletion consistently demonstrated the occurrence of sleepiness 24 hours after the first administration of AMPT in both healthy volunteers (Laruelle et al., 1997; McCann et al., 1992; Verhoeff et al., 2001) and asymptomatic subjects with a history of major depressive disorder (Berman et al., 1999; Hasler et al., 2009).

The current study evaluated the neurobiological correlates of CD-induced daytime sleepiness by measuring the endocrine and cerebral metabolic effects of CD in healthy volunteers and unmedicated RMDD subjects using the serum prolactin concentration and the PET-[¹⁸F]-fluorodeoxyglucose (¹⁸FDG) technique to assess the relationships between neurochemistry, neurophysiology, and the level of sleepiness as assessed by the Stanford Sleepiness Scale.

2. Methods

2.1. Subjects

Individuals ages 18 to 56 years either met DSM-IV criteria for MDD in full remission (n=15) or had no history of any psychiatric disorder and no major psychiatric condition in first-degree relatives (n=13). Diagnosis was established by the Structured Clinical Interview for DSM-IV (First et al., 2001) and confirmed by an unstructured interview with a

psychiatrist. The subjects were recruited through the outpatient clinical services of the NIMH and by advertisements in local newspapers and posters on the NIH campus. Exclusion criteria included major medical illnesses, pregnancy, psychotropic drug exposure (including nicotine) within 3 months, substance abuse within one year, lifetime history of substance dependence, psychiatric disorders other than MDD, or structural brain abnormalities on MRI. Inclusion criteria required that RMDD subjects had remained in remission while off medications 3 months, and manifested depression-onset prior to age 40 years. Written informed consent was obtained as approved by the NIMH IRB.

2.2. Measurement of sleepiness

We applied the Stanford Sleepiness Scale (SSS) to assess AMPT-induced sleepiness. The SSS is a self-rating scale with seven progressive levels of vigilance, and subjects are asked to indicate which level best describes their present state. It has been cross-validated with neuropsychological measures (Hoddes et al., 1973), and mean SSS ratings correlated with performance of the Wilkinson Vigilance Test ($r=0.68$) and with memory performance ($r=0.47$). In addition, the SSS has been shown to be sensitive to the effects of cumulative partial sleep deprivation and recovery oversleeping (Herscovitch and Broughton, 1981). Although designed to measure sleepiness in patients with sleep complaints, it has been shown to be a useful experimental instrument in normal subjects (Curcio et al., 2001).

2.3. Experimental design

Using a randomized, double-blind, placebo-controlled, crossover-design, subjects underwent two identical sessions separated by at least one week, in which they received either AMPT or placebo. To reduce risk for adverse reactions, we used a body weight-adjusted AMPT dose of 40 mg/kg body weight p.o., to a maximum of 4g, over 22 hours. Each session lasted 3 days and were performed on an inpatient basis at the NIH Clinical Center. To reduce the risk of crystalluria during AMPT administration, subjects received sodium bicarbonate, drank 2L of water daily, and underwent urine analysis twice daily. Behavioral ratings included the Stanford Sleepiness Scale (SSS) rated at the time of the PET scan (i.e., 30 hours after the first dose of AMPT or placebo) and additional SSS ratings at 32 and 34 hours after the first dose of AMPT/placebo.

The method of PET imaging and the endocrinological assessment of the AMPT response are detailed in our previous report on the same study (Hasler et al., 2008). Briefly, PET images were obtained 30 hours after administration of the first AMPT/placebo dose, corresponding to the time period when peak behavioral responses were expected (Berman et al., 1999; Bremner et al., 2003). Scanning was performed using a GE Advance scanner in 3D mode (35 contiguous slices, 4.25 mm thick; 3D resolution=6 mm FWHM; GE Medical Systems, Waukesha, WI) and slow bolus (over 2 min) injection of ^{18}F FDG. To obviate the need for *arterial* blood sampling, cerebral glucose utilization was quantified using a method which combines the left ventricular chamber time-tissue radioactivity data, measured using dynamic PET imaging of the heart, with venous blood sampling to provide the ^{18}F FDG input function (Moore et al., 2003; Neumeister et al., 2004). This method has previously been validated against more invasive approaches using arterial plasma sampling (Moore et al., 2003; Neumeister et al., 2004). During image processing, the left ventricular time-radioactivity curve was extended in time to include the time period of the brain emission scan using venous blood samples obtained at 25, 30, 35 and 50 minutes after ^{18}F FDG injection. The mean radioactivity of these samples was divided by the mean left ventricular radioactivity concentration between 25 and 35 minutes post-injection. This ratio was used to scale the 50-minute venous sample concentration, which then was appended to the left ventricular curve to complete the input function used to generate parametric images of

regional cerebral metabolic rates for glucose (rCMRglu), as described by Moore and colleagues (Moore et al., 2003).

To provide an anatomical framework for analysis of the PET images, structural MRI scans were acquired using a 3.0-T scanner. Blood samples were taken 30 hours after the first AMPT intake in each session to measure serum prolactin levels using an electrochemiluminescent immunoassay (Boehringer, Mannheim, Germany).

2.4. Statistical analysis

Full factorial linear mixed models with restricted maximum likelihood estimation were used to examine the effects of drug and diagnosis on SSS scores and serum prolactin concentration. Schwarz's Bayesian criteria were used to determine the best fitting covariance structure for each measure. Pearson correlation was used to look at the relation between sleepiness and serum prolactin concentration.

2.5. PET image analysis

Since the neural AMPT main effect was an increase in metabolism in the anteroventral striatum (AVS), we selected this Region-of-Interest (ROI) to test for an association between the AMPT main effect on brain metabolism and AMPT-induced sleepiness. As in our previous report (Hasler et al., 2008), this ROI was predefined on an anatomical MRI template. Average rCMRglu per voxel was obtained for grey matter voxels within this ROI by multiplying the PET image by a binary mask of gray matter obtained from the segmented anatomical MRI (Neumeister et al., 2004).

To assess correlations between AMPT-induced sleepiness and AMPT-induced metabolic changes across the whole brain, voxel-wise analysis of the PET data was performed using SPM2 in the high-level mathematics environment MATLAB 6.0 (The MathWorks, Inc.; Natick, MA). PET images were co-registered to the MR images and spatially normalized to the Montreal Neurological Institute (MNI) brain template using SPM2. Images were filtered using a 6-mm gaussian smoothing kernel to compensate for anatomical variability and misalignment error arising during spatial normalization. MNI coordinates were non-linearly translated to the stereotaxic spatial array of Talairach and Tournoux (Talairach and Tournoux, 1988) using the MATLAB program min2tal developed by the MRC Cognition and Brain Sciences Unit, Cambridge, UK. The statistical models applied to compare normalized rCMRglu included main effects of placebo versus drug, the behavioral rating of sleepiness, and subject. The significance threshold for the voxel-wise contrasts and correlational analyses was set at $p < 0.001$ for a minimum cluster of 10 voxels (based upon the "expected voxels per cluster" threshold computed by SPM2 for our data set, as provided in the SPM output file).

3. Results

3.1. Subjects

The 13 healthy volunteers (12 females; mean±standard deviation [SD] age=39±12 years) did not differ significantly from the 15 RMDD subjects (14 females; mean±SD age=39±11 years) regarding gender ratio and mean age. Further subject demographics and the behavioral, neural and endocrine responses to CD (AMPT) within the same study population are described in (Hasler et al., 2008).

3.2. CD-induced sleepiness

When examining all sleepiness data (SSS score at 30, 32 and 34 hours after the first dose of AMPT and placebo), there was a significant effect of drug on SSS scores ($F[1,$

49.9]=106.31, $p<0.0001$): sleepiness ratings increased in both groups under AMPT versus placebo (Figure 1). Neither the main effect of time ($F[2, 107.6]=1.52$, $p=0.22$), nor the main effect of diagnosis ($F[1, 29.6]=1.11$, $p=0.30$), nor the interactions between time, drug and diagnosis were significant ($F[2, 105.5]=0.18$, $p=0.83$), indicating a relatively stable drug effect over the time-frame assessed.

The serum prolactin levels increased under AMPT (mean = 7.7 ± 4.0 and 33.3 ± 13.6 $\mu\text{g/l}$ in the baseline and post-treatment conditions, respectively; $F[1, 203]=81.79$, $p<0.0001$). No effect of diagnosis ($F[1, 203]=0.31$, $p=0.58$) or diagnosis-by-treatment interaction ($F[1, 203]=0.00$, $p=0.97$) was evident for the serum prolactin concentrations. The serum prolactin concentrations positively correlated with SSS scores ($r=0.71$, $p<0.0001$), while there was no correlation between serum prolactin and depressive symptoms as assessed by the Montgomery-Asberg Depression Rating Scale ($r=-0.03$, $p=0.69$) or anxiety symptoms as assessed by the Beck Anxiety Inventory ($r=-0.09$, $p=0.20$).

3.3. Correlations between regional cerebral metabolism and sleepiness

Whole brain metabolism did not differ between groups at baseline or between placebo and AMPT conditions ($p>.25$ for main effects of group, main effects of treatment, and group-by-treatment interactions). Regional analyses thus were performed using normalized (regional/global) data to reduce variability introduced by nonspecific fluctuations in global activity. The CD-induced increase in brain metabolism in the AVS-ROI did not correlate with CD-induced sleepiness (right side: $r=-0.32$, $p=0.87$; left side: $r=-0.25$, $p=0.24$).

Table 1 shows the regions in which metabolic changes under AMPT correlated with corresponding changes in sleepiness at $p_{\text{uncorrected}}<0.001$ (voxel-wise analysis using SPM2). There were positive correlations in left parieto-temporal cortex, right superior temporal gyrus, mid-cingulate and posterior cingulate cortex, left insula, bilateral frontal polar cortex (BA 10), right cerebellum, vicinity of periaqueductal gray, right precuneus, left medial orbitofrontal cortex and left ventrolateral prefrontal cortex. Metabolic change and sleepiness correlated negatively at $p_{\text{uncorrected}}<0.001$ in left mid-cingulate cortex (Talairach coordinate $x=-12$, $y=-19$, $z=40$; $t=5.52$) and left anterior cingulate cortex ($x=-6$, $y=30$, $z=21$; $t=3.69$).

In *post hoc* analyses limited to the females (i.e., excluding the male subject from each group) the results were nearly identical. A joint histogram plotting t-scores from the voxel-based correlation analysis of all subjects against only female subjects confirmed that there was no difference in t-scores between the two samples.

4. Discussion

This is the first study to compare effects of CD on daytime sleepiness and brain metabolism. CD induced the same amount of sleepiness in healthy volunteers and in subjects with fully remitted major depressive disorder. CD's neural main effect, the increase in brain metabolism in the anteroventral striatum, did not correlate with CD-induced sleepiness. The most significant positive correlations between AMPT-induced sleepiness and corresponding increases in metabolism appeared in the left lateral temporal cortex, the left insula, the mid-cingulate cortex, the frontal polar cortex, the right cerebellum and the vicinity of the periaqueductal gray. Serum prolactin concentrations were strongly correlated with sleepiness.

Some limitations of our methods merit comment. First, sleepiness was only assessed by a brief self-report scale; more refined measurements of sleepiness including physiological tests would have increased the validity of the findings. Second, the specificity of our results was limited by CD's effects of reducing synthesis of NE as well as DA. Third, while the

PET-FDG technique allowed us to address our primary aim of assessing neurophysiological correlates of CD-induced sleepiness using measures that were unaffected by nonspecific changes on cerebral blood flow and vascular tone, this method could not provide specific biochemical information about catecholamine concentrations. Instead we relied upon the observation that CD produced a consistent rise in serum prolactin, indirectly indicating that catecholamine levels were reduced under AMPT versus placebo (Freeman et al., 2000). Nevertheless, a more selective method for assessing the depth of CD on intrasynaptic DA concentrations would potentially be afforded by PET-[C-11]raclopride measures, which are sensitive to endogenous DA levels (Laruelle et al., 1997). However, such PET imaging methods by be unreliable for estimating tonic dopamine levels in the striatum and extrastriatal regions of healthy humans, which limits its use in CD studies (Cropley et al., 2008). Sleepiness induced by CD may not have the same physiological underpinnings as normal sleepiness or sleepiness caused by sleep deprivation and other sleep-inducing drugs (e.g., benzodiazepines). Fourth, the generalizability of our results was further limited by the predominantly female gender composition of our sample and the history of MDD in more than one-half of the subjects (although we did not find any difference between the diagnostic groups regarding CD-induced sleepiness). Finally, the relationships observed between sleepiness and metabolic change may have reflected relationships between metabolic activity and some other unidentified effect of AMPT that was correlated statistically but not mechanistically with sleepiness.

Because drugs that enhance dopaminergic neurotransmission increase wakefulness and arousal (e.g., psychostimulants), it has been hypothesized that dopamine plays a role in the regulation of the sleep-wake cycle. Potentially consistent with this hypothesis, mice with a deletion of the DA transporter gene showed 20% more wakefulness than control mice and were unresponsive to the wake-promoting effects of psychostimulants (Wisor et al., 2001). The dopaminergic projections from the substantia nigra and ventral tegmental area (VTA) to the striatum, amygdala, and PFC comprise an important modulatory input into these structures (Drevets et al., 1992; Graybiel, 1991). In the striatum, dopaminergic projections synapse onto axon terminals of afferent glutamatergic neurons, and DA release inhibits glutamate release from these neurons (Bamford et al., 2004). Reducing DA input into the striatum thus disinhibits efferent neural transmission from the striatum (Wooten and Collins, 1981). Consistent with this effect, as reported previously (Hasler et al., 2008), the most prominent and consistent neurophysiological effect of CD was the elevation of anteroventral striatal metabolism. Although we did not find correlations between CD-induced metabolic change and sleepiness in the components of the mesostriatal system, such as the striatum, vicinity of the VTA, or substantia nigra, such a relationship was not expected due to similar findings from animal models. Specifically, studies in rats demonstrated that the firing of VTA and substantia nigra neurons projecting to the striatum did not influence behavioral wakefulness (Miller et al., 1983) and that neurotoxic lesions in the VTA did not increase sleepiness (Lai et al., 1999). The strong correlation between serum prolactin levels and sleepiness as well as the absence of a correlation between prolactin and mood and anxiety symptoms suggest that the tuberoinfundibular dopaminergic system, which inhibits prolactin synthesis and release (Moore, 1987), plays a specific role in waking. This is consistent with a recent study in rats demonstrating the participation of dopaminergic cells in the caudal hypothalamus in waking and paradoxical sleep (Leger et al.).

Although the strong correlation between plasma prolactin levels and sleepiness supports the important influence of dopamine on sleepiness in this study, norepinephrine depletion also may have contributed to AMPT's effect on sleepiness. In support of this, the norepinephrine release inhibitor clonidine can produce profound sedation in waking subjects (Carskadon et al., 1989). Historically, the locus coeruleus (LC)-noradrenergic system was referred to as the "ascending reticular activating system" (Berlucchi, 1997), and early speculation regarding

this system's function focused on vigilance and sleep-wake cycle (Sara, 2009). The firing activity of LC neurons occurs at a high frequency during wakefulness, a diminished rate in states of sleepiness and sleep, and ceases during rapid eye movement sleep (Aston-Jones and Bloom, 1981). Furthermore, both noradrenergic and dopaminergic neurons respond to arousing environmental stimuli, and both neurotransmitters act concomitantly to modulate neuronal networks in the prefrontal cortex (Sara, 2009). The arousal-enhancing function of the LC-noradrenergic system has been associated with activation of noradrenergic receptors in the medial preoptic area of the hypothalamus and the medial septal area (Berridge and Waterhouse, 2003). In the current study we were unable to assess the effects of AMPT on metabolic activity in these regions due to their small size relative to the spatial resolution of PET.

We have reported previously that CD induced significantly more depressive and anxiety symptoms in fully remitted subjects with a history of MDD than in controls (Hasler et al., 2008), and that CD had a differential effect on the performance on a reward processing task (Hasler et al., 2009). In contrast, CD induced the same amount of sleepiness in both groups. We found a strong correlation between serum prolactin and sleepiness, while serum prolactin did not correlate with depressive and anxiety symptoms. Taken together, these findings suggest an association between dopamine depletion and sleepiness independent of the brain reward system and the risk for depression. Moreover, one might hypothesize that CD-induced sleepiness is the result of CD's neural main effect, i.e., the dramatic increase in brain metabolism in the anteroventral striatum, which we have previously reported (Hasler et al., 2008). However, the results of this study clearly argues against this hypothesis, given that there was no relationship between CD-induced sleepiness and the CD-induced increase in ventrostriatal metabolism on neither side. As a result, our previously reported imaging findings (Hasler et al., 2008) do not elucidate the neural substrate of sleepiness following CD.

One must be careful about attributing causality while interpreting the correlations between CD-induced sleepiness and CD-induced changes in brain metabolism.. Specifically, there is no indication as to whether sleepiness caused changes in brain activity, changes in brain activity caused sleepiness, or whether they are associated because were induced by CD. CD-induced sleepiness correlated with CD-induced increases in metabolism in the bilateral superior temporal gyrus, the anterior insula, the medial prefrontal cortex, the posterior cingulate gyrus and in the vicinity of the periaqueductal gray. Neuroanatomical experiments in human and nonhuman primates have shown that these regions participate in an extended "visceromotor network" that modulates autonomic, endocrine, emotional, and neurovegetative functions (Ongur et al., 2003). The present findings suggest that the function of this network is modulated by catecholaminergic input to an extent that correlates with subjective sleepiness. The correlation in the vicinity of the periaqueductal gray is particularly interesting given the convergent evidence that this brain region is important in the control of sleep and wake (Lu et al., 2006a; Lu et al., 2006b). The relationship between the metabolic change and sleepiness (midbrain metabolism was positively correlated with sleepiness) may potentially reflect the reduction in the modulatory effect of dopaminergic transmission on glutamate release, which would serve to increase glutamatergic transmission (the regional glucose metabolic signal predominantly is accounted for by glutamatergic transmission) (Hasler et al., 2008; Magistretti et al., 1999). The CD-induced changes in sleepiness ratings also correlated with CD-induced change in metabolism in the vicinity of the cingulate motor area (CMA) in the mid-cingulate cortex. Studies on neuronal activity in the CMA in monkeys suggest that this brain region is involved in the monitoring of movements and in the general intention for action (Hoshi et al., 2005).

In conclusion, CD consistently induced sleepiness in healthy volunteers and subjects with a history of depression. CD-induced sleepiness was paralleled by altered activity in brain structures that formed part of the extended visceromotor network. Correlations between CD-induced sleepiness and CD-induced changes in metabolic activity in the vicinity of the periaqueductal gray suggest that dopamine is involved in the well established sleep/wake controlling function of this brain structure. The close association between serum prolactin and sleepiness points to a potential role of the caudal hypothalamus in the regulation of sleepiness, and the current study encourages further research on the involvement of the dopaminergic system in wakefulness and sleep.

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Figure 1.

Levels of sleepiness at the time of the PET scan classified by drug condition and diagnostic group. Sleepiness was assessed by the Stanford Sleepiness Scale at the time of the PET scan, i.e., 30 hours after the first dose of AMPT or placebo. There was a significant effect of drug ($F[1, 27.1]=46.88$, $p<0.0001$), while there was no effect of diagnosis ($F[1, 27.2]=0.16$, $p=0.69$) and no drug-by-diagnosis interaction ($F[1, 27.1]=0.00$, $p=0.99$). HC=healthy control; rMDD=subjects with remitted major depression; AMPT=alpha-methyl-para-tyrosine. Error bars indicate standard deviations.

Figure 2.

Statistical Parametric Mapping (SPM) image sections illustrating areas where AMPT-induced changes in sleepiness ratings correlated with corresponding changes in regional glucose metabolism in the voxel-wise analysis of the combined subject sample (n=28). Voxels where the p-values exceeded the significance threshold are displayed on orthogonal planes from an anatomical MRI brain image.

Brain regions where glucose utilization correlated with AMPT-induced sleepiness rated by the Stanford Sleepiness Scale.

(A) Correlations in the left orbital prefrontal cortex, the medial prefrontal cortex and the left insula, shown as voxel t-values corresponding to $p < 0.001$.

(B) Correlations in the mid-cingulate cortex, the mesencephalon and the left insula, shown as voxel t-values corresponding to $p < 0.001$.

Stereotaxic coordinates corresponding to the horizontal (x, y) and vertical (z) axes are indicated next to the slides and are interpreted as in table 1. Color bars indicate voxel t values.

Table 1

Regions where catecholamine depletion induced changes in metabolism correlated positively with corresponding changes in sleepiness.

Brain regions	x y z coordinates ^a	Cluster size (voxels)	t-values
L superior temporal gyrus	-51, -2, 2	46	5.07
L anterior insula / ventrolateral PFC	-36, 25, 13	33	4.91
R mid-cingulate gyrus (cingulate motor area)	8, -14, 39	10	4.74
L inferior temporal gyrus	-60, -38, -17	34	4.69
R superior temporal gyrus	60, 0, 7	21	4.58
Ventromedial frontal polar cortex (BA 10)	4, 56, -8	22	4.53
R cerebellum	26, -55, -16	14	4.52
Vicinity of periaqueductal gray	6, -30, -5	25	4.49
L middle temporal gyrus	-48, -10, -8	18	4.21
L medial frontal polar cortex (BA 10)	-4, 64, 4	9	4.15
R frontal polar cortex (BA 10)	14, 61, 8	11	4.11
R Precuneus	6, -74, 44	12	4.10
L middle insula	-46, 4, -4	5	4.05
L medial orbital frontal gyrus	-8, 53, -21	16	3.99
L posterior insula / putamen	-34, -21, 16	5	3.90
L ventrolateral prefrontal cortex	-48, 23, -3	10	3.80
L inferior parietal lobe	-51, -48, 43	6	3.80
Posterior cingulate gyrus	0, -29, 38	5	3.71

The behavioral score for each subject was calculated as the difference in sleepiness as assessed by the Stanford Sleepiness Scale (SSS) at the time of the PET scan between drug condition and placebo condition reflecting the magnitude of catecholamine depletion induced sleepiness. The statistical models for assessing changes in normalized rCMRglu included the main effect of drug versus placebo, the main effect of the sleepiness level, and the main effect of subject. Results shown in the table were significant at $p_{\text{uncorrected}} < 0.001$. No region was identified where the changes in metabolism correlated negatively with the changes in sleepiness. There was no correlation that reached statistical significance after controlling for multiple comparisons.

^a coordinates correspond to the stereotaxic array of Talairach and Tournoux (1988) and denote the distance in mm from the anterior commissure, with positive x = right of midline, positive y = anterior to the anterior commissure, and positive z = dorsal to a plane containing both the anterior and the posterior commissures.