Effects of Yohimbine on Cerebral Blood Flow, Symptoms, and Physiological Functions in Humans

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Objective: Increases in adrenergic activity are associated with stress, anxiety, and other psychiatric, neurological, and medical disorders. To improve understanding of normal CNS adrenergic function, CBF responses to adrenergic stimulation were determined. **Methods:** Using PET, the CBF changes after intravenous yohimbine, an α_2-adrenoreceptor antagonist that produces adrenergic activation, were compared with placebo in nine healthy humans. Heart rate, blood pressure, $Paco_2$, plasma catecholamines, and symptom responses were also determined. **Results:** Among nonscan variables, yohimbine produced significant symptom increases (including a panic attack in one subject), a decrease in $Paco_2$ due to hyperventilation, increases in systolic and diastolic blood pressure, and a trend toward a significant norepinephrine increase. Among scan results, yohimbine produced a significant decrease in whole-brain absolute CBF; regional decreases were greatest in cortical areas. Medial frontal cortex, thalamus, insular cortex, and cerebellum showed significant increases after normalization to whole brain. Medial frontal CBF change was correlated with increases in anxiety. A panic attack produced an increase instead of a decrease in whole-brain CBF. Factors potentially contributing to the observed CBF changes were critically reviewed. Specific regional increases were most likely due in large part to activation produced by adrenergically induced anxiety and visceral symptoms. **Conclusions:** This study supports the relationship of anxiety and interoceptive processes with medial frontal, insular, and thalamic activation and provides a baseline for comparison of normal yohimbine-induced CNS adrenergic activation, adrenergically-based symptoms, and other markers of adrenergic function to stress, emotion, and the adrenergic pathophysiologies of various CNS-related disorders. **Key words:** yohimbine, cerebral blood flow, positron emission tomography, interoception, anxiety, human.

CBF = cerebral blood flow; CNS = central nervous system; PET = positron emission tomography; $Paco_2$ = arterial partial pressure of CO_2.

INTRODUCTION

Adrenergic and noradrenergic changes have been observed in the periphery and in the CNS in association with stress and a number of psychiatric and psychosomatic, as well as medical and neurological, disorders (1–4). (Subsequently, "adrenergic" will refer to noradrenergic as well as adrenergic function.) One way to assess potential adrenergic abnormalities is by administration of substances that modify adrenergic function. Yohimbine, an α_2-adrenoreceptor antagonist that produces adrenergic activation at least to a large extent through its antagonism of inhibitory neuronal autoreceptors (5–7), is one of the substances that has been used. For example, yohimbine has been used to induce anxiety symptoms and study the associated pathophysiological changes, mainly in people with panic disorder (6, 8–11).

One method of evaluating CNS adrenergic function is with functional imaging techniques. Two general methods are available. First, ligands specific for adrenergic receptors could be developed. Second, because coupling exists among neuronal activity, cerebral glucose metabolism (CMRglu), and CBF (12–14), adrenergic function can be assessed by measuring CMRglu or CBF responses to administration of pharmacological agents that act specifically on adrenergic receptor systems. A prior single-photon emission tomography (SPECT) study found that people with panic disorder demonstrated a decrease in the CBF response to yohimbine in the frontal lobe region in comparison to normal subjects (15, 16). A PET study demonstrated that people with posttraumatic stress disorder had decreases in CMRglu in response to yohimbine in several cortical brain regions that showed increases in healthy subjects (17).

In the present study PET was used to determine CBF changes in healthy humans in response to administration of yohimbine. Because effects on CBF are coupled to CNS neuronal activity (12–14), the pattern of CBF response to yohimbine should permit inferences about whole-brain and regional CNS adrenergic function (5–7). Understanding the CBF response in normal subjects should provide a basis for identifying presumptive adrenergic abnormalities in various disorders in sub-

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sequent studies, as well as providing information about pharmacologic mechanisms of adrenergic functioning (1, 18). Prior results (15–17, 19) support the hypotheses that a) yohimbine will decrease whole-brain CBF, b) regional differences in brain CBF response will be observed, especially in cortical areas, c) there will be a significant association between CBF changes and anxiety symptoms in the frontal CNS regions, and d) yohimbine will produce activation in brain regions associated with visceral sensory perceptive processes (20–24).

METHODS

Subjects

Each subject participated in one experimental session of 3 to 4 hours duration. All were free of psychiatric and medical disorders at the time of study, based on a medical history inventory and interview by one of the investigators, and all denied ever having experienced any panic attacks. All denied known history of psychiatric disorders in any first-degree relatives. None were hypertensive, based on sitting blood pressure measurement, and all had normal 12-lead electrocardiograms. All subjects were at least 18 years of age and not older than 40, and all were drug free including avoidance of caffeine for at least 24 hours before study (25). Nine subjects (six women and three men) were studied; mean age was 30.7 years and mean weight was 69.3 kg. All subjects gave written informed consent.

Procedure

Nonscan Procedure. At the beginning of imaging sessions, subjects had two catheters placed, one in an antecubital vein for injection of $[^{15}O]H_2O$ and for obtaining venous blood specimens for measurement of plasma catecholamines (epinephrine and norepinephrine), and the other in the radial artery of the opposite arm at the wrist for measurement of time course of distribution of $[^{15}O]H_2O$ and for obtaining arterial blood for measurement of $Paco_2$. A blood pressure cuff was positioned on the same arm as the venous catheter. Heart rate was determined by radial artery palpation in the same arm as the blood pressure was measured. Drug and placebo were administered intravenously over 3 minutes as a 10-ml bolus. Yohimbine was given in a dose of 0.15 mg/kg, up to a maximum dose of 10 mg, a dose that gave robust effects in prior studies (26, 27). The half-life of intravenous yohimbine is approximately 45 minutes (26), and the peak adrenergic effect at this dose as indicated by plasma norepinephrine changes is within 10 minutes after infusion (27).

Arterial blood for $Paco_2$ was obtained at 2 minutes after the start of the scan. Heart rate and blood pressure were determined immediately after the end of the scan, followed by obtaining a 5-ml venous blood sample for catecholamine determinations. Analog symptom ratings were done immediately after the heart rate and blood pressure measurements and blood sampling. Symptoms, which were rated (0–10, with 0 = "none" and 10 = "most ever"), are listed in Table 1. Subjects were instructed to give an average symptom rating for the duration of the scan. These postscan procedures took approximately 3 minutes to complete.

Blood for catecholamine determinations was collected into tubes containing an antioxidant and anticoagulant. Specimens were stored on ice immediately. Plasma was separated and frozen at −80°C until assay. Assays were performed with HPLC and electrochemical detection (28).

Scans. After catheter insertions and blood pressure cuff positioning, subjects were placed supine in the gantry of the scanner. CBF images were obtained using a Siemens 931/08–12 PET scanner (CTI Inc., Knoxville, TN), which acquires 15 simultaneous, contiguous slices with 6.75-mm slice intervals. Quantitative CBF measurement was performed as follows. After intravenous administration of 80 mCi of $[^{15}O]H_2O$, nine dynamic PET image sets were obtained over 6 minutes and subsequently combined into one set of images representing the aggregate CBF over the 6 minutes. Each image was reconstructed using a filter with a cutoff frequency of 0.45 cycles per projection, giving reconstructed in-plane resolution of 8.0 mm full-width-at-half-maximum (FWHM) and axial resolution of 8.0 mm FWHM. The arterial blood radionuclide concentration was measured continuously (except when the arterial sample for $Paco_2$ was being drawn) from a radial artery using a peristalsis pump and a NE-102 plastic scintillation detector during image acquisition. CBF was calculated by a weighted integral method (29), omitting the first 30 seconds of data, as described previously (30, 31). A two-compartment CBF model with the $[^{15}O]H_2O$ method was used (32). Attenuation correction was performed with a 10 to 15 minute transmission scan using a ^{68}Ge source.

Drug or placebo was administered immediately before the start of each scan. The scan data acquisition started immediately on completion of the infusion. To study each subject in one experimental session and to avoid residual effects of yohimbine during the placebo scan, all subjects received the two PET scans in a single-blind fixed-order design, the first scan after a saline placebo administration and the second after yohimbine. The second scan was always at least 15 minutes after the first, which represents approximately seven half-lives for decay of $[^{15}O]$.

Data Analysis

Using the arterial blood radionuclide time-activity curve, quantitative CBF images were generated. Comparisons of absolute (ie, nonnormalized) CBF after drug vs. after placebo scans were done for whole brain. Absolute CBF values were also used in the region of interest (ROI) and pixel-by-pixel regional analyses (see below).

In addition to absolute CBF analyses, analyses of normalized data were done for both types of regional analyses (33). Normalization to whole brain was used to remove the mean yohimbine or placebo effect on whole-brain CBF. This allows a) comparisons of relative effects on different brain regions to each other after removal of any effects on whole brain, and b) combining data across subjects after removal of expected whole-brain differences in CBF among subjects, thereby reducing variability.

Absolute and normalized regional scan data were analyzed with two methods: a) ROIs drawn in an automated fashion for seven cortical and six subcortical predetermined brain areas and b) with a computer program that determines, on a pixel-by-pixel basis, regions of statistically significant differences between two conditions.

Before both types of analyses, each PET image set was standardized anatomically to a stereotactic atlas brain (34) using a linear scaling edge detection and nonlinear deformation method (35). The spatial location of the ROIs was standardized using a predefined ROI template that followed the same stereotactic orientation and the regional definitions of the atlas. These were irregular ROIs drawn following the contour of the gray matter (one ROI each side) of a stereotactically aligned PET study from a healthy subject by one of the investigators. Cortical areas, except for the cerebellum, thalamus, and putamen, were sampled in this manner at the mid-caudate level, mid-thalamic level. ROIs were then transferred to the study

TABLE 1. Mean (SD) of Nonscan Results

	Scan Condition		Paired *t*	*p* Value
	Placebo	Yohimbine		
Cardiovascular responses				
Heart rate (bpm)	62.8 (7.4)	70.0 (20.2)	1.17	>.1
	62.6 (7.9)	63.8 (8.1)	1.01	>.1
Systolic blood pressure (mm Hg)	119 (7)	128 (14)	2.44	<.05
	119 (7)	126 (8)	2.13	<.08
Diastolic blood pressure (mm Hg)	72.4 (21.0)	84.0 (7.5)	5.67	<.001
	73.4 (6.9)	82.2 (3.8)	4.04	<.006
Plasma catecholamines				
Norepinephrine (pg/ml)	189 (155)	485 (434)	2.50	<.06
	194 (141)	511 (411)	2.30	<.09
Epinephrine (pg/ml)	8.33 (1.26)	23.4 (74.4)	1.14	>.1
	8.00 (0.71)	15.8 (15.8)	1.51	>.1
Respiratory response				
$Paco_2$ (torr)	41.5 (2.6)	38.5 (2.1)	4.24	<.01
	41.6 (2.6)	38.5 (2.1)	4.25	<.01
Symptoms				
Mental anxiety	1.00 (1.41)	5.89 (3.60)	4.05	<.005
	1.00 (1.41)	5.25 (3.81)	3.66	<.01
Physical anxiety	1.22 (3.60)	6.22 (3.12)	5.07	<.002
	1.12 (0.99)	5.75 (2.71)	4.47	<.005
Restlessness	2.00 (1.65)	6.00 (3.75)	3.84	<.006
	2.12 (1.81)	5.50 (2.93)	3.58	<.01
Irritability	1.22 (1.47)	3.56 (3.36)	2.86	<.03
	1.00 (1.41)	2.75 (2.49)	2.70	<.04
Decreased concentration	1.56 (1.68)	5.44 (4.20)	2.74	<.03
	1.50 (1.85)	4.88 (3.72)	2.25	<.06
Relaxed	7.89 (2.31)	0.89 (1.68)	6.73	<.0002
	6.88 (4.05)	1.50 (2.39)	3.74	<.01

Data for all nine subjects are included in the upper line; data without the subject that had the panic attack are in the lower line. Respiratory response ($Paco_2$) was measured 2 minutes after the start of the 6-minute scan. Cardiovascular responses, venous plasma catecholamines, and symptoms (rated 0 = "none" to 10 = "most ever") were rated immediately after the end of the scan. For "mental anxiety," subjects were instructed to rate their cognitive anxiety experience such as apprehension. For "physical anxiety," subjects were instructed to rate their physical anxiety symptoms such as trembling. These postscan procedures required approximately 3 minutes to complete.

images by the same investigator, blind to subject and scan order. The regions chosen for the ROI analysis, with their corresponding Brodmann areas (BA) are listed in Table 2. The frontal and insular cortical ROIs were of particular interest because of results from the prior studies of the effects of yohimbine on frontal CBF (6,15) and CMRglu (17) and because of the putative involvement of the insular cortex in visceral somatosensory processes (see Discussion).

The method for computerized pixel-by-pixel analysis was described previously (36, 37). After reconstruction, all images were realigned to the intercommisural (AC-PC) line. A pixel-by-pixel statistical subtraction analysis between the two scan conditions was performed by estimating the smoothness of the images (38) after three-dimensional Gaussian filtering (FWHM = 9 mm) to enhance the signal-to-noise ratio and compensate for small anatomic variance in the standard stereotactic coordinate system (39). Z-score images were generated using a pooled variance over the cerebral cortex (40). A statistically significant threshold with adjustment for multiple comparisons controlling a Type I error rate at $p = .05$ was estimated on the above Z-score images using a statistical model based on a Euler characteristic (40).

Differences for the ROI analyses were evaluated with paired *t* tests. For the ROI analysis, Spearman rank correlation coefficients were determined for the ROIs with the results of the nonscan variables. For the ROI and correlational analyses, significance levels were defined as $p \leq .05$. For the ROI method, data were analyzed with and without inclusion of the one subject who had the panic attack vs. the other eight subjects (see Results).

RESULTS

Nonscan Results

Results for the nonscan variables are presented in Table 1. Subjective symptom ratings were all significantly changed during the yohimbine scan. One subject, although having no prior history of an anxiety disorder, had a panic attack in response to yohimbine, but was able to lie still to complete the scan. In comparison to the mean for the other subjects, this subject's change in symptoms were "mental anxiety": 10 vs. 4.25; "physical anxiety": 8 vs. 4.62; "restless": 9 vs. 3.38; "irritability": 7 vs. 1.75; "change in concentration": 8 vs. 3.38; and "relaxed": −6 vs. −7.12 (negative

TABLE 2. Mean (SD) of Absolute CBF Scan Results

	Scan Condition		Paired *t*	*p* Value
	Placebo	Yohimbine		
Whole brain	52.5 (5.5)	46.7 (7.7)	2.43	<.05
	52.7 (5.9)	45.1 (6.3)	4.54	<.005
Cortical Areas				
Occipital cortex, bilateral (BA 18)	86.0 (14.3)	76.7 (19.4)	1.90	<.10
	84.8 (14.8)	71.8 (13.5)	2.91	<.03
Parietal cortex, right (BA 39, 40)	72.5 (9.5)	62.8 (8.9)	2.39	<.05
	73.5 (9.5)	61.1 (7.9)	3.89	<.007
Parietal cortex, left (BA 39, 40)	72.7 (9.9)	62.9 (11.4)	2.68	<.03
	72.3 (10.6)	59.8 (7.1)	3.51	<.02
Frontal cortex, right (BA 10)	72.5 (7.7)	66.2 (11.9)	1.63	>.1
	72.8 (8.1)	63.7 (9.9)	3.12	<.02
Frontal cortex, left (BA 10)	72.3 (8.8)	63.6 (11.3)	1.81	>.1
	73.5 (8.6)	61.6 (10.3)	3.54	<.02
Insular cortex, right	81.8 (13.3)	79.6 (16.7)	0.27	>.1
	83.4 (13.2)	77.2 (16.1)	2.04	<.09
Insular cortex, left	80.5 (12.1)	78.4 (15.4)	0.28	>.1
	82.0 (12.1)	76.1 (14.8)	2.05	<.10
Subcortical areas				
Pons, bilateral	58.6 (11.9)	53.0 (13.5)	1.12	>.1
	60.6 (11.3)	52.1 (14.2)	2.25	<.07
Thalamus, bilateral	85.0 (24.1)	80.1 (23.5)	0.43	>.1
	83.6 (25.4)	75.1 (19.3)	1.85	>.1
Putamen, right	79.9 (14.6)	73.4 (14.7)	1.24	>.1
	81.1 (15.1)	72.4 (15.4)	1.97	<.10
Putamen, left	75.5 (12.4)	70.8 (13.1)	1.03	>.1
	77.1 (12.1)	70.3 (13.9)	2.01	<.09
Cerebellum, right	68.7 (8.2)	64.5 (14.9)	0.88	>.1
	69.0 (8.8)	61.4 (12.2)	2.24	<.07
Cerebellum, left	68.7 (8.7)	65.5 (12.2)	0.51	>.1
	69.2 (9.1)	63.1 (10.6)	1.49	>.1

Data for all nine subjects are included; data without the subject that had the panic attack are in the lower line. Absolute CBF (ml/min/100 ml of brain tissue). Bilateral indicates that right and left were averaged together in one regional measurement.

result for "relaxed" because subjects became less relaxed after yohimbine). Because there was no a priori justification for excluding this subject (ie, she had no psychiatric diagnosis before study), she was included in the data analyses. However, to assess the effects of the data from this subject on overall results, the results with the ROI analysis were also analyzed after exclusion of this subject (Tables 1 and 2).

Physiological variables also showed significant effects. $Paco_2$ was significantly decreased after yohimbine. Including all subjects, systolic and diastolic blood pressures both increased significantly, whereas heart rate increased nonsignificantly. Norepinephrine showed a trend toward being increased; epinephrine was minimally increased.

Unlike the other subjects, the subject with the panic attack showed a very large increase in heart rate (56 beats per minute). The overall heart rate increase for all subjects was almost completely due to this subject (mean increase for the other eight subjects was only 1.12 beats per minute). The increase in blood pressure in the subject that panicked was also greater than the mean change for the other subjects (systolic: 30 vs. 6.62 mm Hg; diastolic: 15 vs. 10.1 mm Hg).

Scan Results

Results for the scan variables for the ROI analyses are presented in Table 2 and for the pixel-by-pixel analyses in Figures 1 and 2. Based on the ROI analyses, the eight subjects who did not have a panic attack showed decreases in whole-brain CBF after yohimbine (range of decrease 4%–29%); mean decrease for these eight subjects was approximately 14.5%. The subject who panicked, despite hyperventilating, had a CBF *increase* of approximately 23%; with inclusion of this subject the mean decrease for all nine subjects was approximately 11%.

For both the pixel-by-pixel and the ROI regional analyses, absolute changes and changes of normalized

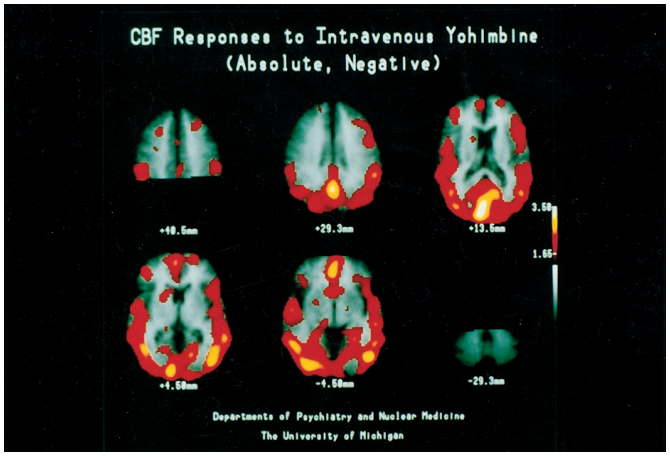

Fig. 1. Z-score significance map of absolute decreases in regional CBF in response to intravenous yohimbine (placebo scan minus yohimbine scan). Scan slices are horizontal at millimeter (mm) levels above (+) or below (−) the AC-PC line as indicated. Difference in each voxel is represented by color coding. Color coding bar indicating correspondence of color to Z-score appears on the Figure (maximum Z-score = 3.50). Right and left are reversed. Map is overlaid on a generic magnetic resonance imaging (MRI) template to provide anatomical orientation.

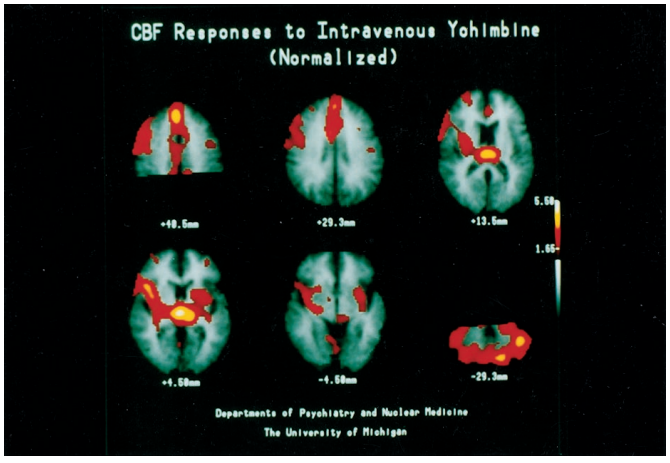

Fig. 2. Z-score significance map of normalized increases in regional CBF in response to intravenous yohimbine (yohimbine scan minus placebo scan). Normalization was performed by dividing each voxel value by whole brain value. Scan slices are horizontal at millimeter (mm) levels above (+) or below (−) the AC-PC line as indicated. Difference in each voxel is represented by color coding. Color coding bar indicating correspondence of color to Z-score appears on the Figure (maximum Z-score = 5.50). Right and left are reversed. Map is overlaid on a generic MRI template to provide anatomical orientation.

data were examined separately. With the pixel-by-pixel analysis, including all nine subjects, the greatest changes in absolute CBF were decreases in cortical areas (Figure 1); no regions showed significant absolute increases (not shown). After normalization, no areas were decreased significantly more than whole brain (not shown). There were relative increases in the thalamus, medial frontal area, insular cortices bilaterally, and the cerebellum (Figure 2). (Note that the frontal ROI includes both frontal and prefrontal areas and part of the anterior cingulate region.)

For the ROI analyses, with inclusion of all subjects, there were statistically significant decreases in absolute CBF in both right and left parietal cortices; all other analyzed regions also decreased, but not significantly, and the decreases in the insular cortices were very small (Table 2). After normalization, there were significant decreases (ie, significantly greater than whole brain) in the left frontal cortex and the right parietal cortex (both $p < .03$, not shown). For normalized data, the right and left insular cortices, the thalamus, and the cerebellum showed significant increases (ie, lesser decreases than whole brain, all $p < .02$, not shown). Thus, the pixel-by-pixel and ROI analyses were in agreement for both absolute and normalized data. The fact that with the ROI analysis frontal cortices were decreased more than whole brain, whereas with the pixel-by-pixel analysis medial frontal cortex specifically was decreased less than whole brain, reflects the fact that the medial frontal region is only a relatively small part of the whole frontal area, an area that had a different CBF response than the remainder of the overall frontal region.

With the ROI analysis, after exclusion of the subject who had the panic attack, for absolute values, five of the seven cortical regions (right and left frontal and parietal cortices and occipital cortex bilaterally) were significantly decreased in comparison to whole brain, and the pons and right cerebellum showed trends (Table 2). After normalization, with exclusion of the subject who panicked, left frontal and right parietal cortices again were significant (ie, decreased more than whole brain, both $p < .04$, not shown). Again, the right and left insular cortices, the thalamus, and the cerebellum showed significant increases (ie, a lesser decrease than whole brain, all $p < .03$, not shown).

The subject who panicked showed a qualitatively somewhat different CBF response from the other subjects; in this subject, the left frontal cortex, the pons, and the cerebellum were decreased less than whole brain, whereas in the other subjects, the decreases in these areas were larger than whole brain. These results for the subject who panicked represented Z-score values above the mean of 1.09 (pons), 1.18 (left cerebellum), 1.50 (left frontal cortex), and 1.62 (right cerebellum). Thus, although suggestive of a qualitatively different pattern, none were outside of the 95% confidence intervals.

Correlations were performed between the regions

found to be significant in the ROI analysis (bilateral thalamus and medial frontal cortex, and right and left insulae and cerebellar cortices) with the hemodynamic, $Paco_2$, catecholamine, and symptom variables. Using normalized scan data, the relative increase in medial frontal cortical CBF was significantly positively correlated with change in heart rate ($r = +0.81$, two-tailed $p < .05$) and change in "mental anxiety" ($r = +0.68$, two-tailed $p = .05$); the magnitude of the correlation with change in heart rate was due mainly to the subject who had the panic attack, but was comparable for "mental anxiety" with or without inclusion of that subject. For absolute data, the correlation for medial frontal cortex with heart rate was +0.66 and with "mental anxiety" was +0.31; these correlations did not reach statistical significance. No other correlations for absolute or normalized data reached statistical significance.

Because of the small sample size, to reach significance the absolute value of an individual correlation was at least ±0.68. Looking for trends of correlations that did not reach statistical significance but were nonetheless suggestive of patterns of possible physiological importance, correlations more than +0.50 were assessed for each of the six brain regions found to be significant in the normalized data. For absolute scan data, each of the ROIs analyzed was positively correlated with $Paco_2$ and epinephrine. Additionally, thalamus, medial frontal cortex, and right insula were positively correlated with heart rate and irritability, whereas the two cerebellar cortices and the left insula were positively correlated with systolic blood pressure and negatively correlated with difficulty concentrating. For normalized scan data, medial frontal cortex was positively correlated with restlessness and $Paco_2$ (as well as heart rate and mental anxiety), and negatively correlated with feeling relaxed. The cerebellar cortices were negatively correlated with norepinephrine and $Paco_2$ and positively with feeling relaxed. A few other sporadic correlations exceeded ±0.50, but no pattern was suggested. Interpretation of these correlations must be done cautiously because of the small sample sizes and multiple tests performed.

DISCUSSION

The hypotheses presented in the Introduction based on previous research were supported. In response to CNS and systemic adrenergic activation by yohimbine, whole-brain CBF was decreased, with the greatest decreases in cortical regions. The correlational data demonstrated an association between anxiety and changes in medial frontal cortex. Brain regions associated with visceral sensory processes—thalamus and insular cortices, as well as medial frontal cortex region—were affected by yohimbine-induced adrenergic activation. Additionally, the correlational patterns suggest relationships among epinephrine and $Paco_2$ with absolute changes in yohimbine-induced CBF changes, as well as an overall responsivity of the medial frontal cortex to both physiological and symptom variables.

Potential Effects of Panic, Hypocapnia, Habituation, and Physiological Changes

There are several possible factors that could affect the results observed. Inclusion of the subject that panicked had only a minor effect. The decrease in absolute whole-brain CBF was more robust if this subject was not included, but for normalized data, the pattern of significant results was not changed. It is noteworthy that the occurrence of a panic attack increased whole-brain CBF despite the multiple other factors that tended to decrease it. For the correlational results, the significant relationship for medial frontal cortical CBF with anxiety was not affected by inclusion of the subject who panicked, whereas the relationship with heart rate was.

$Paco_2$ was reduced during the scans involving the active substance because the subjects hyperventilated. It is well documented that hypocapnia reduces CBF (41), including studies with functional imaging techniques (42–44). Thus, whole-brain CBF decreases seen in response to yohimbine were due at least in part to hyperventilation. However, effects of hypocapnia seem to be uniform throughout the brain gray matter (44, 45). Thus, significant effects seen in normalized data only in specific regions are unlikely to be due to hypocapnia.

Because the yohimbine scan for each subject was completed after the placebo scan (ie, fixed-order, single-blind design), another possible cause for the reduction in whole-brain CBF during the second scan is habituation. A number of studies have reported decreases in whole-brain CBF from the first to subsequent scans (46–51), although some studies did not report this (52–54). Based on these results, as with hypocapnia, habituation might have contributed to the whole-brain CBF decrease, but does not account for the specific regional increases seen after normalization.

Among other nonscan variables, potential causes of the CBF changes might include changes in heart rate, blood pressure, and norepinephrine. The heart rate and blood pressure changes could not account for the change, however, because they should be associated with CBF increases rather than decreases. It also seems unlikely that an increase in norepinephrine contrib-

uted to the CBF decrease. Brain norepinephrine has been associated with cerebral vasoconstriction (19), although not all data are consistent with this (55–58). Data on the effects of circulating norepinephrine on CBF are inconsistent (59); circulating norepinephrine does not cross the blood-brain barrier (60–62). CBF was not decreased by intracarotid injections of norepinephrine in humans (63).

Potential Vascular, Metabolic, and CNS α_2-Adrenoreceptor Distribution Effects

The above factors could have accounted for some or all of the whole-brain CBF decrease, but are unlikely to account for the relative increases seen in medial frontal cortex, insular cortices, thalamus, and cerebellar cortices. Factors that could have produced these specific regional effects include vascular and other potential adrenoreceptor-mediated effects and symptoms including anxiety and visceral symptoms.

Although yohimbine could have vascular effects without entering the CNS, it does freely enter mammalian brain (64). Results from prior studies of the effects of yohimbine on CBF and cerebral vasculature (15, 16, 65, 66) are inconsistent, but suggest that yohimbine decreases CBF in some brain regions. In humans, yohimbine decreased frontal cortical CBF in people with panic disorder (15, 16); other areas studied—parietal, temporal, and visual cortices, striatum, thalamus, and cerebellum—did not show differences. This study, however, only assessed diagnosis-related differences, not absolute effects of yohimbine. The present study found yohimbine-induced CBF decreases in cortical regions. Prior studies of adrenergically activating agents on regional CBF (67–69) did not find CBF changes in frontal cortex or cerebellum different from other regions, although thalamus did seem less sensitive (insular cortex was not tested). Thus, direct adrenergic effects of yohimbine on cerebral vasculature does not explain the results observed.

The observation that clonidine, an α_2-adrenoreceptor *agonist,* reduces whole-brain CBF (70–73), as did yohimbine, an *antagonist*, indicates that adrenoreceptor-mediated effects also cannot fully account for the observed results; if it did, clonidine should produce changes in CBF opposite from yohimbine. In a human PET study with clonidine (74), increased CBF was observed mainly in subcortical regions, whereas decreases occurred mainly in cortical regions. In conjunction with our results, these findings are inconsistent with a single reciprocal mechanism.

Effects of yohimbine on CMRglu have also been addressed (17, 75). In humans, yohimbine produced increases in whole-brain CMRglu and in cortical and subcortical structures (17). The fact that yohimbine increases CMRglu implies that it produces neuronal activation (12–14). Thus, regions found to be different from whole brain in this study were different due to neuronally-mediated activation, not just smaller CBF decreases. In other words, differences in regional pattern between the effects of yohimbine on CBF and CMRglu indicates that yohimbine had specific regional effects over and above direct effects on CBF.

As noted in the Introduction, it seems likely that effects of yohimbine observed in this study were due at least partly to direct activation of CNS α_2 adrenoreceptors. Does the distribution of α_2 adrenoreceptors in human brain parallel the pattern of effects seen in the present study? Previous studies showed highest levels of human brain α_2 adrenoreceptors in neocortex, cingulate gyrus, hippocampus, and hypothalamus. Regions with very low levels included thalamus and white matter. Cerebellum, amygdala, midbrain, pons, medulla, and basal ganglia were intermediate (76–78). Comparison of receptor distribution to the pattern of regional CBF effects in this study indicates that CBF changes were not simply due to receptor activation by yohimbine. Although in the present study, yohimbine produced effects on several cortical areas, there were also effects on CBF in thalamus and possibly cerebellum, regions with only low or intermediate numbers of receptors. Thus, neither vascular nor CNS α_2-adrenoreceptor-mediated effects can explain the pattern of regional increases observed.

Anxiety and Visceral Sensory Symptoms

Yohimbine produces symptoms associated with adrenergic activation, including subjective anxiety, especially in anxiety disorders. Although no completely consistent CNS abnormalities have been observed, in imaging studies of anxiety frontal cortical changes have been reported (15, 16, 79–84). The results of the present study, including correlational results, are consistent with this association of anxiety with frontal changes. These results are also consistent with prior findings that CBF and CMRglu changes in frontal (85–91) and other areas (87, 89, 92) are associated with emotional experiences in normal subjects. Production of subjective sensations probably played a significant role in the yohimbine-induced effects observed in this study.

A major issue in understanding the pathophysiologies of psychosomatic and psychiatric disorders and emotion is the relationship between visceral sensory experiences and CNS function. For example, what is the mechanism of awareness of heart action during a panic attack? Not a great deal is known about CNS

mechanisms of visceral sensory awareness (interoception) (20–24), although studies have investigated afferent function of the autonomic nervous system (23, 93–95). Animal studies indicate that anatomic regions involved in visceral afferent function include brain stem structures, hypothalamus, amygdala, thalamus, and cortex—especially the insular cortex (93, 94).

The insula is strongly implicated in visceral (and somatic, Ref. 96) sensory function. The insular cortex is involved in affective components of visceral function: a) insula demonstrates extensive connections with limbic structures, including amygdala (97–100), b) functional connections between insula and viscera exist (94, 97, 100, 101), c) the insula is involved in cardiac control (102–104), "stress" (105), the "fight-or-flight" response (106), and "sudden death" (107), and d) insular activation occurred in CBF studies of panic disorder and phobias (80, 81, 108, 109) and with emotion in normal subjects (89). These observations are consistent with important insular involvement in normal and abnormal visceral awareness.

Other structures are implicated. The locus coeruleus, which is comprised of adrenergic cell bodies, is involved in visceral sensory processes (110), of special relevance because of the known activation of the locus coeruleus by yohimbine (5–7) and because of involvement of CNS adrenergic functioning in attentional processes—of essential importance in awareness, including visceral awareness, and in anxiety (111–112). Finally, the thalamus is involved in sensory processes, including visceral sensory processes (113–115).

Consistent with the above, in the present study yohimbine activated not only frontal cortex but also insular cortices and thalamus (Figure 2). Although vascular and receptor effects might have contributed to yohimbine-induced regional changes, it is likely that these frontal, insular, and thalamic changes were produced mainly by adrenergic activation leading to CNS-mediated anxiety-like reactions and sensations mediated through visceral sensory pathways. Thus, interoceptive processes probably played an important role in the effect observed. This study provides support for involvement of thalamus and insular and frontal cortices in visceral sensory-perceptual processes.

CONCLUSIONS

Symptoms and physiological effects of yohimbine observed here are consistent with results from prior studies. Scan data demonstrate that yohimbine produced decreases in whole-brain CBF, which was more prominent cortically than subcortically. Multiple mechanisms that could have contributed to these decreases are reviewed. Relative increases in CBF superimposed on the decreases were observed in medial frontal cortex, insular cortex, thalamus, and cerebellar hemispheres. The explanation for these increase most consistent with the pattern of activated regions observed is that these changes were produced by adrenergically induced increases in anxiety and visceral symptom perception. This study of yohimbine-induced CBF changes provides further support for the association of visceral sensations, anxiety, and emotion with the particular brain regions that were activated.

These results advance understanding of how adrenergic mechanisms control CBF and thus CNS function. It provides a baseline for studying how these processes might be dysfunctional in various disorders. Pharmacological activation can provide an experimental model to study normal and pathological interoceptive processes and improve understanding of the relationships among CNS adrenergic function, regional CBF and CMRglu, and brain mechanisms of emotion and visceral sensation.

REFERENCES

1. Cameron OG. Adrenergic dysfunction and psychobiology. Washington DC: American Psychiatric Press; 1994.
2. Cameron OG, Smith CB, Nesse RM, Hill EM, Hollingsworth PJ, Abelson JA, Hariharan M, Curtis GC. Platelet α_2-adrenoreceptors, catecholamines hemodynamic variables, and anxiety in panic patients and their asymptomatic relatives. Psychosom Med 1996;58:289–301.
3. Lake CR, Ziegler MG. The catecholamines in psychiatric and neurologic disorders. Boston: Butterworth Publishers; 1985.
4. Ziegler MG, Lake CR. Norepinephrine. In: Wood JH, Brooks BR, editors. Frontiers in clinical neuroscience. Vol 2. Baltimore: Williams and Wilkins; 1984.
5. Goldberg, MR, Robertson, Yohimbine D. A pharmacological probe of the α_2 adrenoreceptor. Pharmacol Rev 1983;35: 143–80.
6. Redmond DE. New and old evidence for the involvement of a brain norepinephrine system in anxiety. In: Fann WE, Karacan I, Porkorny AD, Williams RL, editors. Phenomenology and treatment of anxiety. New York: SP Medical and Scientific Books; 1979. p. 153–203.
7. Smith CB, Hollingsworth PJ. Classification of adrenergic receptors. In: Cameron OG, editor. Adrenergic dysfunction and psychobiology. Washington DC: American Psychiatric Press; 1994. p. 73–87.
8. Abelson JL, Cameron OG. Adrenergic dysfunction in anxiety disorders. In: Cameron OG, editor. Adrenergic dysfunction and psychobiology. Washington DC: American Psychiatric Press; 1994. p. 403–46.
9. Charney DS, Heninger GR, Breier A. Noradrenergic function in panic anxiety: effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. Arch Gen Psychiatry 1984;41:751–63.
10. Charney DS, Woods SW, Goodman WK, Heninger GR. Neurobiological mechanisms of panic anxiety: biochemical and behavioral correlates of yohimbine-induced panic attacks. Am J Psychiatry 1987;144:1030–6.

11. Charney DS, Woods SW, Krystal JH, Nagy LM, Heninger GR. Noradrenergic neuronal dysregulation in panic disorder: the effects of intravenous yohimbine and clonidine in panic disorder patients. Acta Psychiatr Scand 1992;86:273–82.
12. Roland PE. Brain activation. New York: Wiley-Liss; 1993. p. 469–504.
13. Villringer A, Dirnagl U. Optical imaging of brain function and metabolism 2. New York: Plenum; 1997. p. 155–220.
14. Jueptner M, Weiller C. Review: does measurement of regional cerebral blood flow reflect synaptic activity? Implication for PET and fMRI. Neuroimage 1995;2:148–56.
15. Woods SW, Koster K, Krystal JK, Smith EO, Zubal IG, Hoffer PB, Charney DS. Yohimbine alters regional cerebral blood flow in panic disorder. Lancet 1988;2:678.
16. Woods SW. Regional cerebral blood flow imaging with SPECT in psychiatric disease: focus on schizophrenia, anxiety disorders, and substance abuse. J Clin Psychiatry 1992;53(Suppl): 20–5.
17. Bremner JD, Innis RB, Ng CK, Staib LH, Salomon RM, Bronen RA, Duncan J, Southwick SM, Krystal JH, Rich D, Zubal G, Dey H, Soufer R, Charney DS. Positron emission tomography measurement of cerebral metabolic correlates of yohimbine administration in combat-related posttraumatic stress disorder. Arch Gen Psychiatry 1997;54:246–54.
18. Heal DJ, Marsden CA. The pharmacology of noradrenaline in the central nervous system. New York: Oxford University Press; 1990.
19. Wahl M, Schilling L. Regulation of cerebral blood flow: a brief review. Acta Neurochirica 1993;59(Suppl):3–10.
20. Chernigovskiy VN. Interoceptors. Washington DC: American Psychological Association; 1967.
21. Reed SD, Harver A, Katkin ES. Interoception. In: Cacioppo JT, Tassinary LG, editors. Principles of psychophysiology: physical, social, and inferential elements. Cambridge: Cambridge University Press; 1990. p. 253–91.
22. Vaitl D. Interoception. Biol Psychol 1996;42:1–27.
23. Cervero F, Morrison JFB. Visceral sensation: progress in brain research. Vol 67. Amsterdam: Elsevier; 1986.
24. Adam G. Visceral perception: understanding internal cognition. New York: Plenum Press; 1998.
25. Cameron OG, Modell JG, Hariharan M. Caffeine and human cerebral blood flow: a positron emission tomography study. Life Sci 1990;47:1141–6.
26. Guthrie SK, Hariharan M, Grunhaus LJ. Yohimbine bioavailability in humans. Eur J Clin Pharmacol 1990;39:409–11.
27. Guthrie SK, Grunhaus L, Pande AC, Hariharan M. Noradrenergic response to intravenous yohimbine in patients with depression and comorbidity of depression and panic. Biol Psychiatry 1993;34:558–61.
28. Cameron OG, Gunsher S, Hariharan M. Venous plasma epinephrine levels and the symptoms of stress. Psychosom Med 1990;52:411–24.
29. Alpert NM, Eriksson L, Chang JY, Bergstrom M, Litton JE, Correia JA, Bohm C, Ackerman RH, Taveras JM. Strategy for the measurement of regional cerebral blood flow using short-lived tracers and emission tomography. J Cereb Blood Flow Metab 1984;4:28–34.
30. Koeppe RA, Holden JE. Performance comparison of parameter estimation techniques for the quantitation of local cerebral blood flow by dynamic positron computed tomography. J Cereb Blood Flow Metab 1985;5:224–34.
31. Koeppe RA, Hutchins GD, Rothley JM, Hichwa RD. Examination of assumptions for local cerebral blood flow studies in PET. J Nucl Med 1987;28:1695–703.
32. Huang S, Phelps ME. Principles of tracer kinetic modeling in positron emission tomography and autoradiography. In: Phelps ME, Mazziotta JC, Schelbert HR, editors. Positron emission tomography and autoradiography. New York: Raven Press; 1986. p. 287–346.
33. Fox PT, Mintun MA, Raichle ME, Herscovitch P. A noninvasive approach to quantitative functional brain mapping with $H_2(^{15})O$ and positron emission tomography. J Cereb Blood Flow Metab 1984;4:329–33.
34. Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain. New York: Thieme Medical Publishers; 1988.
35. Minoshima S, Koeppe RA, Frey KA, Ishihara M, Kuhl DE. Stereotactic PET atlas of the human brain: aid for visual interpretation of functional brain images. J Nucl Med 1994;35: 949–54.
36. Minoshima S, Koeppe RA, Fessler JA, Mintun MA, Berger KL, Taylor SF, Kuhl DE. Integrated and automated data analysis method for neuronal activation studies using ^{15}O-water PET. In: Uemura K, Lassen NA, Jones T, Kanno I, editors. Quantification of brain function, tracer kinetics and image analysis in brain PET. Amsterdam: Excerpta Medica (Elsevier) 1993; 409–17.
37. Minoshima S, Koeppe RA, Mintun MA, Berger KL, Taylor SF, Frey KA, Kuhl DA. Automated detection of the intercommisural line for stereotactic localization of functional brain images. J Nucl Med 1993;34:322–9.
38. Friston KJ, Frith CD, Liddle PF, Frackowiack RSJ. Comparing functional PET images: the assessment of significant changes. J Cereb Blood Flow Metab 1991;11:690–9.
39. Adler RJ, Hasofer AM. Level crossings for random fields. Annals of probability 1976;4:1–12.
40. Worsley KJ, Evans AC, Marrett S, Neelin P. A three-dimensional analysis of CBF activation studies in human brain. J Cereb Blood Flow Metab 1992;12:900–18.
41. Grubb RL, Raichle ML, Eichling JO. The effects of changes of $Paco_2$ on cerebral blood volume, blood flow, and vascular mean transit time. Stroke 1974;5:630–9.
42. Bednarczyk EM, Rutherford WF, Leisure GP, Munger MA, Panacek EA, Miraldi FD, Green JA. Hyperventilation-induced reduction in cerebral blood flow: assessment by positron emission tomography. DICP, Ann Pharmacother 1990;24:456–9.
43. Momose T, Sasaki Y, Nishikawa J, Watanabe T, Nakashima Y, Katayama S, Sano I, Nakajima T, Ohtake T, Takashiro K, Watanabe E. Functional brain studies with H_2-^{15}O-PET: strategies and problems for approaching higher brain functions with H_2-^{15}O-PET. Radiat Med 1991;9:122–6.
44. Ramsey SC, Murphy K, Shea SA, Friston KJ, Lammertsma AA, Clark JC, Adams L, Guz A, Frackowiak RSJ. Changes in global cerebral blood flow in humans: effect of regional cerebral blood flow during a neural activation task. J Physiol 1993;471: 521–34.
45. Mathew RJ, Wilson WH. Cerebral blood flow changes induced by CO_2 in anxiety. Psychiatry Res 1988;23:285–94.
46. Risberg J, Maximilian AV, Prohovnik,I. Changes in cerebral activation patterns during habituation to mental testing. Acta Neurol Scand Suppl 1977;64:266–7.
47. Warach S, Gur RC, Gur RE, Skolnick BE, Obrist WD, Reivich M. The reproducibility of the ^{133}Xe inhalation technique in resting studies: task order and sex related effects in healthy young adults. J Cereb Blood Flow Metab 1987;7:702–8.
48. Warach S, Gur RC, Gur RE, Skolnick BE, Obrist WD, Reivich M. Decreases in frontal and parietal lobe regional cerebral blood flow related to habituation. J Cereb Blood Flow Metab 1992;12: 546–53.

49. Seitz RJ, Roland PE. Variability of the regional cerebral blood flow pattern studied with [^{11}C]-fluoromethane and positron emission tomography (PET). Comput Med Imaging Graph 1992; 16:311–22.
50. Schoning M, Scheel P. Color duplex measurement of cerebral blood flow volume: intra- and interobserver reproducibility and habituation to serial measurements in normal subjects. J Cereb Blood Flow Metab 1996;16:523–31.
51. Rajah MN, Hussey D, Houle S, Kapur S, McIntosh AR. Task-independent effect of time on rCBF. Neuroimage 1998;7: 314–25.
52. Ingvar M, Eriksson L, Greitz T, Stone-Elander S, Dahlbom M, Rosenqvist G, af Trampe R, von Euler C. Methodological aspects of brain activation studies: cerebral blood flow determined with [^{15}O] butanol and positron emission tomography. J Cereb Blood Flow Metab 1994;14:628–38.
53. VanMeter JW, Maisog JM, Zeffiro TA, Hallett M, Herscovitch P, Rapaport SI. Parametric analysis of functional neuroimages: application to a variable-rate motor task. Neuroimage 1995;2: 273–83.
54. Bench CJ, Frith CD, Grasby PM, Friston KJ, Paulesu E, Frackowiak RSJ, Dolan RJ. Investigations of the functional anatomy of attention using the Stroop test. Neuropsychologica 1993;31: 907–22.
55. Dahlgren N, Lindvall O, Sabake T, Stenevi U, Siesjo BK. Cerebral blood flow and oxygen consumption in the rat brain after lesions of the noradrenergic locus coeruleus system. Brain Res 1981;209:11–23.
56. Kushinsky W, Suda S, Bunger R, Yaffe S, Sokoloff L. The effects of intravenous norepinephrine on the local coupling between glucose utilization and blood flow in the rat brain. Pflugers Arch 1983;398:134–8.
57. Tuor UI, Edvinsson L, McCullock J. Catecholamines and the relationship between cerebral blood flow and glucose use. Am J Physiol 1986;251:H824–33.
58. Myburgh JA, Upton RN, Grant C, Martinez A. A comparison of the effects of norepinephrine, epinephrine, and dopamine on cerebral blood flow and oxygen utilisation. Acta Neurochirica 1998;71(Suppl):19–21.
59. Harik SI. Catecholaminergic innervation of cerebral blood vessels. In: Phillis JW, editor. The regulation of cerebral blood flow. Boca Raton FL: CRC Press; 1993. p. 79–95.
60. Oldendorf WH. Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. Am J Physiol 1971; 221:1629–39.
61. MacKenzie ET, McCulloch J, O'Keane M, Pickard JD, Harper AM. Cerebral circulation and norepinephrine: relevance of the blood-brain barrier. Am J Physiol 1976;231:483–8.
62. Hardabo JE, Owman C. Barrier mechanisms for neurotransmitter monoamines and their precursors at the blood-brain barrier. Ann Neurol 1980;8:1–11.
63. Olesen J. The effect of intracarotid epinephrine, norepinephrine, and angiotensin on the regional cerebral blood flow in man. Neurology 1972;22:978–87.
64. Ho AKS, Hoffman DB, Gershon S, Loh HH. Distribution and metabolism of tritiated yohimbine in mice. Arch Int Pharmacodyn 1971;194:304–15.
65. Linder J. Cerebral and ocular blood flow during α_2-blockade: evidence for a modulated sympathetic response. Acta Physiol Scand 1981;113:511–7.
66. Toda N. Alpha adrenergic receptor subtypes in human, monkey and dog cerebral arteries. J Pharmacol Exp Ther 1983;226: 861–8.
67. Edvinsson L, Lacombe P, Owman C, Reynier-Rebuffel AM, Seylaz J. Quantitative changes in regional cerebral blood flow or rats induced by alpha- and beta-adrenergic stimulants. Acta Physiol Scand 1979;107:289–96.
68. Gulati A, Srimal RC, Dhawan BN. Alteration in systemic hemodynamics and regional brain blood flow by isoprenaline. Indian J Med Res 1988;88:265–72.
69. Edvinsson L, MacKenzie ET, McCulloch J. Cerebral blood flow and metabolism. New York: Raven Press; 1993. p. 183–230.
70. Bertel O, Marx BE, Conen D. Effects of antihypertensive treatment on cerebral perfusion. Am J Med 1987;82(Suppl 38): 29–36.
71. Bolme P, Forsyth RP, Ishizaki T, Melmon KL. Hemodynamic effects of systemic and central administration of clonidine in the monkey. Am J Physiol 1975;228:1276–9.
72. James IM, Larbi E, Zaimis E. The effect of the acute intravenous administration of clonidine (St 155) on cerebral blood flow in man. Br J Pharmacol 1970;39:198P–9P.
73. Kanawati IS, Yaksh TL, Anderson RE, Marsh RW. Effects of clonidine on cerebral blood flow and the response to arterial CO_2. J Cereb Blood Flow Metab 1986;6:358–65.
74. Fu C, Kapur S, Houle S, Brown GM. Localization of the functional effects of clonidine by PET (Abstract). Biol Psychiatry 1995;37:653.
75. Savaki HE, Kadekaro M, McColluch J, Sokoloff L. The central noradrenergic system in the rat: metabolic mapping of alpha-adrenergic blocking agents. Brain Res 1982;234:65–79.
76. Maloteaux JM. Drug and transmitter receptors in human brain: characterization and localization of serotonin, dopamine and adrenergic receptors. Acta Neurol Belg 1986;86:61–129.
77. Pascual J, del Arco C, Gonzalez AM, Pazos A. Quantitative light microscopic autoradiographic localization of α_2-adrenoceptors in the human brain. Brain Res 1992;585:116–27.
78. Berkowitz DE, Price DT, Bello EA, Page SO, Schwinn DA. Localization of messenger RNA for three distinct α_2-adrenergic receptor subtypes in human tissues: evidence for species heterogeneity and implications for human pharmacology. Anesthesiology 1994;81:1235–44.
79. De Cristofaro MTR, Sessarego A, Pupi A, Biondi F, Favarelli C. Brain perfusion abnormalities in drug-naive, lactate-sensitive panic patients: a SPECT study. Biol Psychiatry 1993;33: 505–12.
80. Rauch SL, Savage CR, Alpert NM, Miguel EC, Baer L, Bretter HC, Fischman AJ, Manzo PA, Moretti C, Jenike MA. Positron emission tomographic study of simple phobic symptom provocation. Arch Gen Psychiatry 1995;52:20–8.
81. Rauch SL, van der Kolk BA, Fisler RE, Alpert NM, Orr SP, Savage CR, Fischman AJ, Jenike MA, Pitman RK. A symptom provocation study of posttraumatic stress disorder using positron emission tomography and script-driven imagery. Arch Gen Psychiatry 1996;53:380–7.
82. Mindus P, Ericson K, Greitz T, Meyerson BA, Nyman H, Sjogren I. Regional cerebral glucose metabolism in anxiety disorders studied with positron emission tomography before and after psychosurgical intervention. Acta Radiol Suppl 1986; 369:444–8.
83. Nordahl TE, Semple WE, Gross M, Mellman TA, Stein MB, Goyer G, King AC, Uhde TW, Cohen RM. Cerebral glucose metabolic differences in patients with panic disorder. Neuropsychopharmacology 1990;3:261–72.
84. Zubieta JK, Chinitz JA, Lombardi U, Fig LM, Cameron OG, Liberzon I. Medial frontal cortex involvement in PTSD symptoms: A SPECT study. J Psychiatr Res 1999;33:259–64.
85. George MS, Ketter TA, Parekh PI, Horwitz B, Herscovitch P,

Post RM. Brain activity during transient sadness and happiness in healthy women. Am J Psychiatry 1995;152:341–51.
86. Partiot A, Grafman J, Sadato N, Wachs J, Hallett M. Brain activation during the generation of nonemotional and emotional plans. Neuroreport 1995;6:1269–72.
87. Schneider F, Gur RE, Mozley LH, Smith RJ, Mozley PD, Censite DM, Alavi A, Gur RC. Mood effects on limbic blood flow correlate with emotional self-rating: a PET study with oxygen-15 labeled water. Psychiatry Res Neuroimaging 1995;61: 265–83.
88. Lane RD, Reiman EM, Ahern GL, Schwartz GE, Davidson RJ. Neuroanatomical correlates of happiness, sadness, and disgust. Am J Psychiatry 1997;154:926–33.
89. Reiman EM, Lane RD, Ahern GL, Schwartz GE, Davidson RJ, Friston KJ, Yun LS, Chen K. Neuroanatomical correlates of externally and internally generated human emotion. Am J Psychiatry 1997;154:918–25.
90. Zald DH, Pardo JV. Emotion, olfaction, and the human amygdala: amygdala activation during aversive olfactory stimulation. Proc Natl Acad Sci USA 1997;94:4119–24.
91. Lane RD, Reiman EM, Bradley MM, Lang PJ, Ahern GL, Davidson RJ, Schwartz GE. Neuroanatomical correlates of pleasant and unpleasant emotions. Neuropsychologica 1997;35: 1437–44.
92. Ketter TA, Andreason PJ, George MS, Lee C, Gill DS, Parekh PI, Willis MW, Herscovitch P, Post RM. Anterior paralimbic mediation of procaine-induced emotional and psychosensory experiences. Arch Gen Psychiatry 1996;53:59–69.
93. Cechetto DF. Central representation of visceral function. Federation Proc 1987;46:17–23.
94. Cechetto DF, Saper CB. Role of the cerebral cortex in autonomic function. In: Loewy AD, Spyer KM, editors. Central regulation of autonomic functions. New York: Oxford University Press; 1990. p. 208–23.
95. Freire-Maia L, Azevedo AD. The autonomic nervous system is not a purely efferent system. Med Hypotheses 1990;32:91–9.
96. Casey KL, Minoshima S, Berger KL, Koeppe RA, Morrow TJ, Frey KA. Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. J Neurophysiol 1994;71:802–7.
97. Mesulam MM, Jones EJ. The insula of Reil in man and monkey. In: Peters A, Jones EJ, editors. Cerebral cortex. Vol 4. Association and auditory cortices. New York: Plenum Press; 1985. p. 179–226.
98. Kapp BS, Schwaber JS, Driscoll PA. The organization of insular cortex projections to the amygdaloid central nucleus and autonomic regulatory nuclei of the dorsal medulla. Brain Res 1985; 360:355–60.
99. Saper CB. Convergence of autonomic and limbic connections in the insular cortex of the rat. J Comp Neurol 1982;210: 163–73.
100. Augustine JR. Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Rev 1996;22: 229–44.
101. Loewy AD, Spyer KM. Central regulation of autonomic functions. New York: Oxford University Press; 1990.
102. Oppenheimer SM, Cechetto DF. Cardiac chronotropic organization of the rat insular cortex. Brain Res 1990;533:66–72.
103. Oppenheimer SM, Gelb A, Girvin JP, Hachinski VC. Cardiovascular effects of human insular cortex stimulation. Neurology 1992;42:1727–32.
104. Ruggiero DA, Mraovitch S, Granata AR, Anwar M, Reis DJ. A role of insular cortex in cardiovascular function. J Comp Neurol 1987;257:189–207.
105. Cechetto DF. Identification of a cortical site for stress-induced cardiovascular dysfunction. Integr Physiol Behav Sci 1994;29: 362–73.
106. Jansen ASP, Nguyen XV, Kerpitsky V, Mettenleiter TC, Loewy AD. Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. Science 1995;270: 644–6.
107. Oppenheimer SM, Wilson JX, Guiraudon C, Cechetto DF. Insular cortex stimulation produces lethal cardiac arrhythmias: a mechanism of sudden death? Brain Res 1991;550:115–21.
108. Reiman EM, Raichle ME, Robins E, Butler FK, Herscovitch P, Fox P, Perlmutter J. The application of positron emission tomography to the study of panic disorder. Am J Psychiatry 1986;143:469–77.
109. Reiman EM, Raichle ME, Robins E, Mintun MA, Fusselman MJ, Fox PT, Price JL, Hackman, KA. Neuroanatomical correlates of a lactate-induced anxiety attack. Arch Gen Psychiatry 1989;46: 493–500.
110. Svensson TH. Peripheral, autonomic regulation of locus coeruleus noradrenergic neurons in brain: putative implications for psychiatry and psychopharmacology. Psychopharmacology 1987;92:1–7.
111. Arnsten AFT, Steere JC, Hunt RD. The contribution of $alpha_2$-noradrenergic mechanisms to prefrontal cortical cognitive function. Arch Gen Psychiatry 1996;53:448–55.
112. Robbins TW. Arousal and attentional processes. Biol Psychol 1997;45:57–71.
113. Newman J. Thalamic contributions to attention and consciousness. Conscious Cogn 1995;4:137–58.
114. Kawakita K, Dostrovsky JO, Tang JS, Chaing CY. Responses of neurons in the rat thalamic nucleus submedius to cutaneous muscle and visceral nociceptive stimuli. Pain 1993;55: 327–38.
115. Ruggiero DA, Anwar S, Kim J, Glickstein SB. Visceral afferent pathways to the thalamus and olfactory tubercle: behavioral implications. Brain Res 1998;799:159–71.