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In Vivo *Imaging of Nitrous Oxide-induced Changes in Cerebral Activation during Noxious Heat Stimuli*

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Background: **Although previous studies have provided some insight into the pharmacologic aspects of nitrous oxide analgesia, the neural circuits mediating its antinociceptive effect remain relatively unexplored. Positron emission tomography was used in nine volunteers to identify the loci of nitrous oxide–modulated cerebral responses to a peripheral noxious stimulus.**

Methods: **Nitrous oxide–pain interactions were studied by comparing regional cerebral blood flow responses to a 48°C tonic heat stimulus, applied to each volunteer's left forearm, during room air inhalation with those obtained while 20% nitrous oxide was administered. Two cerebral blood flow scans were obtained with the ^{15}O-water technique during each condition. Locations of specific regional activation related to pain, and nitrous oxide, were identified using the statistical parametric mapping method, with a significance level of $P < 0.01$. Pain was rated by visual analog scale and the values were compared using Wilcoxon rank sum analysis.**

Results: **Pain produced cerebral activation in the contralateral thalamus, anterior cingulate, and supplementary motor area. Adding nitrous oxide during pain stimulation abolished activation in these areas but was associated with activation in the contralateral infralimbic and orbitofrontal cortices. In parallel, mean visual analog scale scores decreased significantly from 67 ± 4 (SEM) to 54 ± 5 ($P < 0.05$).**

Conclusions: **Nitrous oxide, at 20% concentration, appears to modulate pain processing in the brain's medial pain system, and also activates the infralimbic and orbitofrontal cortices. The potential contribution of the affected brain areas to nitrous oxide analgesia is discussed. (Key words: Analgesia. Anesthetics, inhalation: nitrous oxide. Brain: cerebral blood flow. Measurement techniques: tomography, emission-computed. Pain. Radionuclide imaging: ^{15}O-water.)**

THE antinociceptive effects of a low concentration of nitrous oxide (*i.e.*, 20%) have been exploited for many years in the practice of dentistry, obstetrics, and emergency medicine. Recent work addressing the mechanisms of nitrous oxide analgesia suggests that some interaction may exist between nitrous oxide and endogenous opioid peptides. Animal studies have demonstrated nitrous oxide–induced met-enkephalin and β-endorphin release,[1] and in humans at least some of nitrous oxide's antinociceptive effect has been shown to be reversible by naloxone.[2] Endogenous opioid peptide release, however, has not been confirmed in human studies, largely because of methodologic difficulties posed by the invasiveness of the measurement technique.[3] Although such studies may suggest at least one neurochemical mediator for nitrous oxide analgesia, they do little to reveal the neural networks that participate in the antinociceptive response.

Because the pain experience is so complex, human studies focusing on the ultimate variable of interest—the perception of pain—are important. According to contemporary pain theories, the various components of nociception, such as sensory-discriminative and affective-motivational, involve different brain areas.[4] Positron emission tomography (PET) has proved to be a sensitive tool for mapping the net functional effects of a stimulus or drug on regional neuronal activity in the living human brain. For example, PET studies on humans have found that noxious peripheral thermal stimuli evoke highly localized cerebral activation responses

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in the anterior cingulate cortex, thalamus, supplementary motor area, and primary and secondary somatosensory cortices.[5,6] Because nociceptive processing seems to involve distinct brain areas, it is reasonable to assume that analgesic drugs also exert their effect in a highly localized manner. Positron emission tomography also has shown that systemic morphine- and fentanyl-induced pain relief is associated with activation in the anterior cingulate, prefrontal cortex, and caudate nucleus.[7-9] These observations suggest that a site of pain–opioid interaction is the anterior cingulate, the cortical projection of the medial pain system that is primarily responsible for processing the affective-motivational components of pain.[4] In contrast, more recent *in vivo* opioid receptor imaging by PET directly demonstrates a high density of opiate binding sites in the thalamus, cingulate and prefrontal cortices, with a relative lack of binding in the somatosensory cortices,[10] where the lateral pain system projects.[4]

To test the hypothesis that the medial pain system is the common site of action of the pharmacologically dissimilar systemic analgesics, nitrous oxide and opioids, we used PET to compare changes in regional cerebral synaptic activity induced by a pain stimulus, with those accompanying the combined administration of a pain stimulus and 20% nitrous oxide inhalation. Such a relatively low concentration of nitrous oxide does not uncouple regional cerebral blood flow (rCBF) and glucose metabolism ($rCMR_{glu}$),[11] unlike higher concentrations,[12] allowing rCBF measurements with ^{15}O-water to reflect regional synaptic activity with greater validity.[13-15]

Table 1. Experimental Conditions and Data Analysis

Experimental conditions	
Pain-Nitrous Oxide Experimental Group	
[Control]	Room air inhalation focusing on crosshair
[Nitrous Oxide]	Nitrous oxide inhalation focusing on crosshair
[PS]	Room air inhalation focusing on crosshair pain stimulation
[PS + Nitrous Oxide]	Nitrous oxide inhalation focusing on crosshair pain stimulation
Visual control group	
[Control]	Room air inhalation focusing on crosshair
[Nitrous Oxide]	Nitrous oxide inhalation focusing on crosshair
[VS]	Room air inhalation visual stimulation
[VS + nitrous oxide]	Nitrous oxide inhalation visual stimulation
Data analysis	
Comparisons (subtractions)	Activated brain areas related to:
Pain-Nitrous Oxide Experimental Group	
[PS] – [Control]	Pain stimulation
[Nitrous Oxide] – [control]	Nitrous oxide inhalation
[PS + Nitrous Oxide] – [Nitrous Oxide]	Pain stimulation in the presence of nitrous oxide
[PS + Nitrous Oxide] – [PS]	Nitrous oxide inhalation during pain stimulation
Visual control group	
[VS] – [control]	Visual stimulation
[VS + Nitrous Oxide] – [Nitrous Oxide]	Visual stimulation in the presence of nitrous oxide

PS = pain stimulation; VS = visual stimulation.

Materials and Methods

Participants

Nine right-handed healthy volunteers (six women, three men), ages 22 to 44 yr (mean, 28.5 yr), gave written informed consent. All procedures were approved by the University of Pittsburgh Institutional Review Board (IRB #931063) and Radioactive Drug Review Committee. Participants were screened for neurological, psychiatric, and substance abuse disorders. All the women had negative results of serum pregnancy tests on the day of the PET scan.

Experimental Design

Volunteers underwent PET scanning in two groups, each under four experimental conditions (table 1). Five participants (Pain–Nitrous Oxide Experimental Group) received a 48°C tonic, noxious, thermal pain stimulus ([PS] condition), produced by a thermostatically controlled 2 × 2 cm aluminum hot plate (Omega Cn7600 Thermostat, Stanford, CT) applied to the participant's left (nondominant) volar forearm. This thermal stimulator maintained the preset temperature throughout the stimulus duration of 2 min. During the control condition ([Control]), volunteers inhaled room air; during the nitrous oxide condition ([Nitrous Oxide]), a mixture of 20% nitrous oxide and 30% oxygen was administered. The nitrous oxide-modulated pain stimulation condition ([PS + Nitrous Oxide]) consisted of the simultaneous administration of the tonic pain stimulus and nitrous oxide. In each experimental condition, all volunteers

fixed their gaze at a crosshair on an overhead computer screen (table 1).

Nitrous oxide is known to increase global CBF in a dose-dependent manner[16]; such neuronal activity-independent global CBF increases could alter or obscure any activation-induced rCBF changes. Thus, to ensure that cerebral vascular responsiveness was not blunted during the nitrous oxide experimental condition, four participants underwent PET scanning during a visual activation paradigm known to elicit a maximal cerebral activation response in the visual cortex (Visual Control Group).[17] The participants' gaze remained fixed on the crosshair in each experimental condition except during visual stimulation. During the visual stimulation condition ([VS]), participants viewed red and black annular segments reversed at a frequency of 6 Hz, which evokes an rCBF increase of maximal intensity in the occipital cortex.[17] A nitrous oxide–modulated visual stimulation condition ([VS + Nitrous Oxide]) consisted of the combination of nitrous oxide administration and visual stimulation (table 1).

Regional CBF was measured using the positron-labeled tracer ^{15}O-water as an indicator of regional synaptic activity.[13,14] Two scans were obtained in each condition, for both the Pain–Nitrous Oxide Experimental and Visual Control groups. The order of scans was randomized and counterbalanced to control for any possible order effects. However, control conditions always preceded nitrous oxide conditions to avoid residual drug effects. Quantitative psychophysical ratings of pain stimuli were made on a 100-mm visual analog scale by each participant, both 5 s after application of and again 5 s before removal of the pain stimulus. Zero denoted no sensation, 50 denoted the pain threshold, and 100 signified severe pain.

Positron Emission Tomography Scanning Procedures

Positron emission tomography scans were obtained on an ECAT 951R/31 system (Siemens Medical Systems, Hoffman Estates, IL), which provides 31 parallel slices over an axial field of view of 10.8 cm, with a transverse and axial image resolution of approximately 6 mm full-width half-maximum (FWHM). Volunteers were positioned and the PET gantry rotated and tilted such that the lowest imaging plane was approximately parallel to and 1 cm superior to the canthomeatal line. To correct for positron emission attenuation by the intra- and extracerebral tissues, a transmission scan was obtained using an external positron emitting rod source (^{68}Ge/^{68}Ga). Reconstruction of PET images from the obtained data resulted in scans of 128 × 128 pixels, with dimensions of 2.05 × 2.05 mm.

For each rCBF scan, a 50-mCi intravenous bolus injection of ^{15}O-water was used. Twenty seconds after the injection, a 60-s data acquisition was started[13] with at least 12 min between each scan to allow the tracer concentration to decay to background levels. Pain stimulation ([PS] and [PS + Nitrous Oxide]) conditions in the Pain–Nitrous Oxide Experimental Group and visual ([VS] and [VS + Nitrous Oxide]) conditions in the Visual Control Group began 15 s after the tracer injection and continued for 65 s. Nitrous oxide administration began approximately 15 min before PET scanning in the [Nitrous Oxide] and [PS + Nitrous Oxide], and [Nitrous Oxide] and [VS + Nitrous Oxide] conditions, to establish a steady-state brain concentration. Nitrous oxide dilutions (10 l/min total gas flow) were delivered through an anesthesia machine (Modulus II Plus; Ohmeda, Madison, WI) *via* tight-fitting face mask and semiclosed breathing circuit. An end-tidal nitrous oxide concentration of 20% was confirmed with a multiple-gas analyzer (Rascal II; Ohmeda, Salt Lake City, UT). Other physiologic monitors included noninvasive blood pressure cuff, electrocardiograph, pulse oximeter, and capnograph.

Data Analysis and Statistics

All PET images were reconstructed and registered among participants to correct for any head movement that occurred during scan collection.[18] Resulting data were analyzed separately using the statistical parametric mapping software.[19,20] During this process, images are averaged within and across participants for each experimental condition to facilitate the detection of experimental condition-related rCBF changes with enhanced sensitivity. Interparticipant image averaging, however, mandates the normalization of brain position, size, and shape differences. Therefore, image sets from each volunteer were transformed onto the stereotactic atlas of Talairach and Tournoux[21] by an automated routine that first determines the intercommissural (AC—PC) line, rotates the PET data to match the axis, and then proportionately stretches the PET image sets to fit the atlas. Then, to reduce image noise and accommodate interparticipant differences in cerebral anatomy and function, a Gaussian filter was applied to all image data (20 mm FWHM). Differences in global ^{15}O-water activity among participants and conditions were normalized by analysis of covariance, with global activity as an inde-

pendent variable and regional activity as a dependent variable.[19] With this correction, inter- and intraparticipant variations in activity due to global CBF cannot obscure regional changes. The result of this process is a mean regional activity that is linearly related to the actual rCBF value and associated variance for every pixel during each experimental condition. Comparisons of rCBF between conditions were performed on a pixel-by-pixel basis using *t* statistics. Regions where pixels reached probability values less than 0.01 were considered significant. To reach significance, pixels also had to be in a contiguous group extending over more than one transverse plane, to provide protection against type I error, as demonstrated by the lack of rCBF changes in same-state comparisons. Z values were calculated by transforming the *t* statistic to a unit Gaussian distribution to facilitate comparisons of experimental groups of different size. The results were also displayed in coronal, transverse, and sagittal views of the brain.

The two groups of participants were analyzed separately. In the Pain–Nitrous Oxide Experimental Group, to identify brain areas of significant rCBF increases (activation) related to pain stimulation; nitrous oxide inhalation; pain stimulation in the presence of nitrous oxide; and nitrous oxide inhalation during pain stimulation, the image comparisons (subtractions) listed in table 1 were made. First, in the Visual Control group, areas that showed significant rCBF increases in the occipital cortex during visual stimulation in the absence ([VS] − [Control]) and presence ([VS + Nitrous Oxide] − [Nitrous Oxide]) of nitrous oxide were identified with statistical parametric mapping.[19,20] Then, to assess nitrous oxide's effect on cerebral vasculature responsiveness, in the identified areas the magnitude of visual stimulation-related rCBF increases, measured in the absence ([VS]) and presence of nitrous oxide ([VS + Nitrous Oxide]), was compared, *post hoc*, using the point analysis tool of the statistical parametric mapping software.[19,20] Specifically, in areas of visual activation-related rCBF changes, mean percentage rCBF increases in randomly selected pixels of the occipital cortex during nitrous oxide ([VS + Nitrous Oxide]) *versus* room air inhalation ([VS]), were analyzed for differences with *t* statistics at a significance level of 0.05.

Vital sign data were averaged for all participants in each condition and processed using analysis of variance to detect any significant ($P < 0.05$) differences between experimental conditions. Visual analog scale scores were analyzed nonparametrically using the Wilcoxon rank sum test.

Results

All participants completed their respective group's protocol and tolerated the PET scanning procedures well. The peripheral thermal stimulus was rated as painful by all participants, as reflected in the mean visual analog scale score of 67 ± 4 mm (SEM). Inhalation of 20% nitrous oxide during the thermal stimulus ([PS + Nitrous Oxide] condition) resulted in a significant decrease in the mean visual analog scale score to 54 ± 5 by the Wilcoxon rank sum test ($P < 0.03$), although the stimulus was still rated as painful. Vital signs did not change significantly during the experiments (table 2).

Comparison of scans obtained during room air inhalation alone to those acquired during pain stimulation ([PS] − [Control]) showed significant rCBF increases in the contralateral thalamus, anterior cingulate cortex (area 24), and supplementary motor area when compared with control ($P < 0.01$; fig. 1, table 3). The [Nitrous Oxide] − [Control] comparison revealed significant rCBF increases in the anterior cingulate cortex bilaterally (areas 24, 32; vertical extent relative to AC-PC line: 0 to 20 mm; $P < 0.01$; table 4). Comparison of rCBF during simultaneous pain stimulation and nitrous oxide inhalation with that during nitrous oxide inhalation alone (*i.e.*, [PS + Nitrous Oxide] − [Nitrous Oxide]), revealed significant rCBF increases in the infralimbic area (area 25) and orbitofrontal cortex (areas 10, 11), contralateral to the side of pain stimulus (fig. 2, table 5). The previously demonstrated pain-related activations were not found in the presence of nitrous oxide. This is demonstrated by the nonsignificant percentage rCBF increases in pixels located in the thalamus, anterior cingulate, and supplementary motor area (table 5) and in figure 2, where the nitrous oxide effect has been subtracted from the pain-plus-nitrous oxide condition. This subtraction logically should leave only pain-related activations, because the common nitrous oxide effects are subtracted from the comparison process.

Comparison of rCBF scans obtained during pain stimulation with those during simultaneous pain stimulation and nitrous oxide inhalation ([PS + Nitrous Oxide] − [Pain]) revealed activation in the infralimbic area (area 25), orbitofrontal cortex (areas 10, 11), and anterior cingulate cortex (area 24; vertical extent relative to AC-PC line: 0 to 20 mm). Areas 25 and 11 were activated contralateral to the pain stimulus (fig. 3, table 6). Activation of area 24 in the right hemisphere did not overlap with the pain-activated subregion of area 24.

Table 2. Vital Signs

	[Control]	[Nitrous Oxide]	[VS]	[VS + Nitrous Oxide]	[PS]	[PS + Nitrous Oxide]
Mean systemic blood pressure (mmHg)	78 ± 6	74 ± 8	75 ± 4	73 ± 5	79 ± 5	76 ± 7
Heart rate (beats/min)	68 ± 8	65 ± 6	68 ± 8	70 ± 6	72 ± 7	69 ± 7
Respiratory rate (breaths/min)	13 ± 4	12 ± 4	12 ± 4	13 ± 3	14 ± 3	14 ± 5
Peripheral oxygen saturation (%)	99 ± 1	99 ± 2	99 ± 1	99 ± 1	99 ± 1	99 ± 1
End-tidal carbon dioxide tension (mmHg)	40 ± 2	39 ± 2	39 ± 2	40 ± 2	38 ± 3	39 ± 3

Values are mean ± SEM (n = 4 for the [VS] and [VS + Nitrous Oxide] conditions; n = 5 for the [Control], [Nitrous Oxide], [PS], and [PS + Nitrous Oxide] conditions) measured before and after the experimental conditions. For all vital signs, no significant differences were noted between control and any other condition ($P < 0.05$).

PS = pain stimulation; VS = visual stimulation.

Visual stimulation-related percentage rCBF increases in randomly selected pixels of the occipital cortex during nitrous oxide inhalation ([VS + Nitrous Oxide]) did not differ significantly from those measured while participants breathed room air ([VS]) ($P > 0.05$; table 7).

Discussion

Our results show that in healthy humans, inhalation of 20% nitrous oxide abolishes the pain-evoked responses in the contralateral thalamus, anterior cingulate cortex, and supplementary motor area, and activates the infralimbic (area 25) and orbitofrontal cortices (areas 10, 11; fig. 2, table 5).

The pain activation ([PS] − [Control]) results of this study (fig. 1, table 3) correspond with the findings of previous investigations.[5,6] Activation in the thalamus and anterior cingulate cortex (area 24) in response to peripheral pain stimulation, as we report, has been a consistent finding in these studies.[5,6] Electrophysiologic recordings indicate that nociceptive information

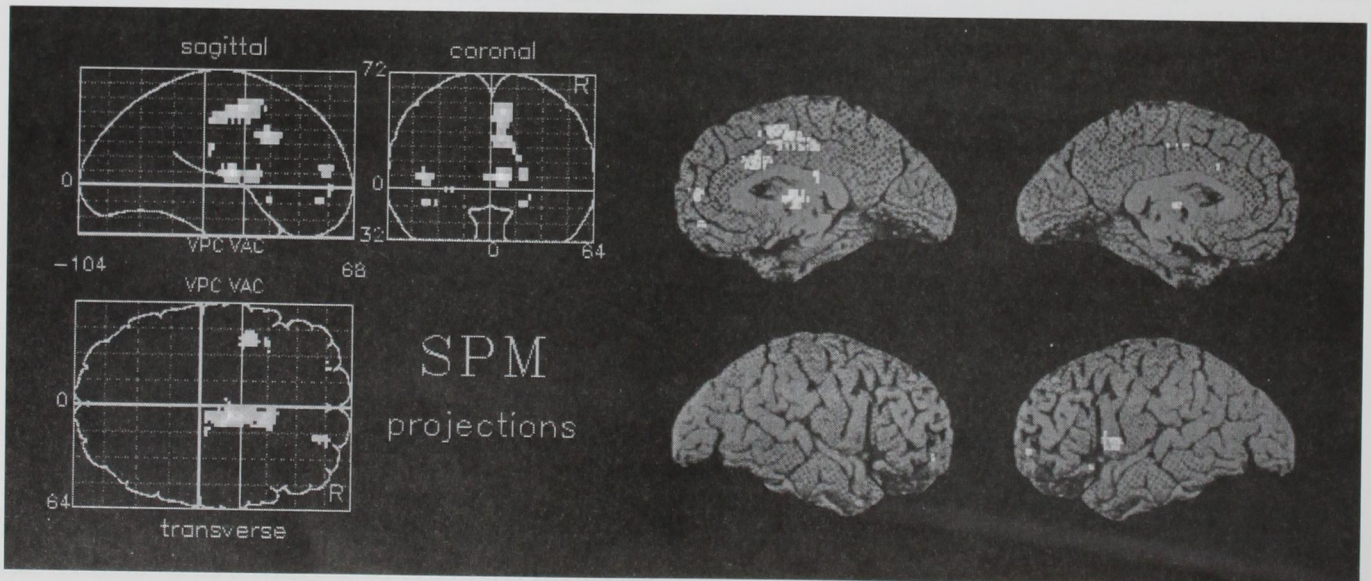

Fig. 1. Statistical parametric maps of neuronal activation during pain stimulation alone ([PS] − [Control]). Pixels that are significant at the given threshold of $P < 0.01$ are displayed on single sagittal, coronal, and transverse projections of the brain as lighter shades of gray, where the lightest shade indicates the greatest degree of activation (see table 3 for anatomic locations). R = right hemisphere; L = left hemisphere; VAC = vertical line through the anterior commissure; VPC = vertical line through the posterior commissure.

Table 3. Areas of rCBF Increases during Pain Stimulation ([PS] − [Control])

Region	Brodmann's Area	Coordinates			Percentage rCBF Increase	z-Score
		x	y	z		
Thalamus (R)		8	−6	4	2.7	2.643
Thalamus (R)		10	−8	8	2.8	3.051
Thalamus (R)		4	−4	12	2.7	2.501
Anterior cingulate (R)	24	8	16	36	2.0	2.568
Anterior cingulate (R)	24	10	−14	40	2.7	2.415
Anterior cingulate (R)	24	4	4	40	2.3	2.451
Supplementary motor area (R)	6	6	14	48	2.7	2.381
Supplementary motor area (R)	6	10	6	52	2.0	2.372

Only areas with a z-score > 2.326 ($P < 0.01$) are listed. The coordinates in a standard stereotactic space (Talairach and Tournoux, 1988) are given (in millimeters) for the maximally significant pixel in each SPM-identified area.

Percent rCBF values are mean ± SEM (n = 4) as described in Materials and Methods.

x = lateral displacement from the midline (− for the left hemisphere); y = anteroposterior displacement relative to the anterior commissure (− for positions posterior to the latter); z = vertical position relative to the AC-PC line (− if below this line); PS = pain stimulation; R = right.

reaches the anterior cingulate *via* direct projections from the medial thalamic nuclei.[22] Clinical observations of patients with chronic pain after anterior cingulotomy underscore its importance in the affective-motivational aspects of pain.[23] Another brain region where significant pain-related activation was found is the supplementary motor area contralateral to the noxious stimulus. Human PET studies have shown significant supplementary motor area activation during movement planning.[24] Thus the activation observed in our study is consistent with pain-related initiation of withdrawal from the noxious stimulus, although none of the participants actually moved during the study.

The [PS + Nitrous Oxide] − [Nitrous Oxide] (fig. 2, table 5) comparison revealed that pain stimulation, in the presence of nitrous oxide, was not associated with the activation observed in the thalamus, anterior cingulate cortex, and supplementary motor area during pain stimulation in the absence of nitrous oxide ([PS] − [Control]; fig. 1, table 3). The [PS + Nitrous Oxide] − [Nitrous Oxide] comparison, however, showed new areas of activation in the infralimbic (area 25) and orbitofrontal cortices (areas 10, 11) contralateral to the pain stimulus. Additional brain areas activated during simultaneous pain stimulation and nitrous oxide inhalation are revealed in the [PS + Nitrous Oxide] − [PS] comparison and include bilateral anterior cingulate activation in the same small regions as in the [Nitrous Oxide] − [Control] comparison (fig. 3, tables 4, 6). This indicates that pain stimulation during nitrous oxide inhalation does not alter the activation pattern evoked by nitrous oxide alone. The different pain activation pattern in the presence rather than the absence of nitrous oxide, however, suggests that nitrous oxide affects cerebral nociceptive processes.

Alternatively, the lack of pain-related response in

Table 4. Areas of rCBF Increases during Nitrous Oxide Inhalation Alone ([Nitrous Oxide] − [Control])

Region	Brodmann's Area	Coordinates			Percentage rCBF Increase	z-Score
		x	y	z		
Anterior cingulate (L)	24	−6	30	0	3.8	3.146
Anterior cingulate (R)	24	8	32	0	3.2	2.525
Anterior cingulate (L)	24	−14	40	4	3.4	2.553
Anterior cingulate (R)	24	6	40	4	3.2	2.590
Anterior cingulate (L)	24	−6	32	16	2.7	2.599
Anterior cingulate (R)	24	10	26	20	3.0	2.767

Only areas with a z-score > 2.326 ($P < 0.01$) are listed. Details regarding the stereotactic coordinates are as in table 3.
L = left; R = right.

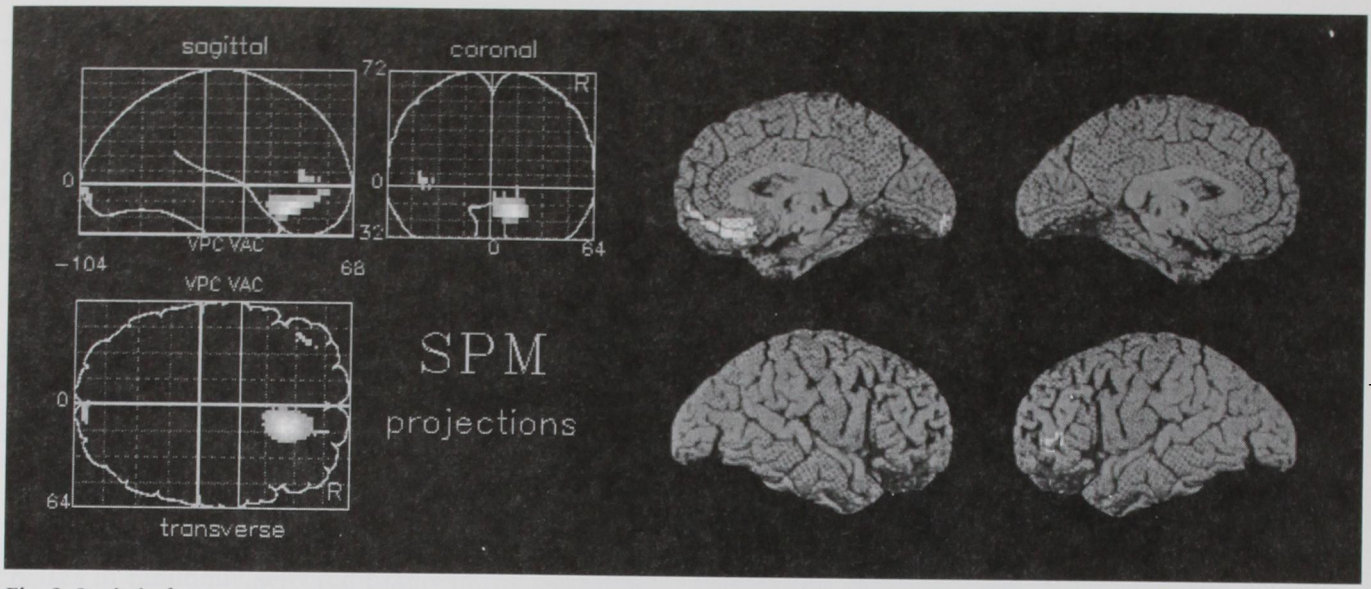

Fig. 2. Statistical parametric maps of neuronal activation during pain stimulation in the presence of nitrous oxide ([PS + Nitrous Oxide] − [Nitrous Oxide]). The activation pattern reflects nitrous oxide-modulated pain responses because only activations associated with nitrous oxide alone ([Nitrous Oxide]) are subtracted from the image obtained during the combined administration of pain and nitrous oxide ([PS + Nitrous Oxide]). Consequently, if nitrous oxide had no effect on pain processing, this activation pattern would have been identical to that in figure 1. Regional brain activation is represented as described in figure 1, and the relevant anatomic assignments are specified in detail in table 5. See figure 1 for explanations of views and abbreviations.

the thalamus, anterior cingulate, and supplementary motor area during nitrous oxide inhalation could be due to the fact that nitrous oxide, administered in the absence of pain, might have evoked activation in the same brain areas that pain evoked activation, thus the [PS + Nitrous Oxide] − [Nitrous Oxide] subtraction would have eliminated apparent pain-related activations. However, this is not the case because our [Nitrous Oxide] − [Control] comparison shows that the nitrous oxide-related activations are

Table 5. Areas of rCBF Increases during Pain Stimulation in the Presence of Nitrous Oxide ([PS + Nitrous Oxide] − [Nitrous Oxide])

Region	Brodmann's Area	Coordinates			Percentage rCBF Increase	z-Score
		x	y	z		
Infralimbic area (R)	25	12	36	−16	4.2	3.743*
Orbitofrontal cortex (R)	11	10	36	−12	4.1	3.576*
Orbitofrontal cortex (R)	10	18	48	−8	3.2	2.539*
Orbitofrontal cortex (R)	10	16	52	−4	3.0	2.480*
Thalamus (R)		8	−6	4	0.2	NS
Thalamus (R)		10	−8	8	0.0	NS
Thalamus (R)		4	−4	12	−0.2	NS
Anterior cingulate (R)	24	8	16	36	0.4	NS
Anterior cingulate (R)	24	10	−14	40	−0.3	NS
Anterior cingulate (R)	24	4	4	40	0.8	NS
Supplementary motor area (R)	6	6	14	48	0.6	NS
Supplementary motor area (R)	6	10	6	52	0.9	NS

Coordinates are as in table 3.

NS = nonsignificant; PS = pain stimulation; L = left; R = right.

* $P < 0.01$.

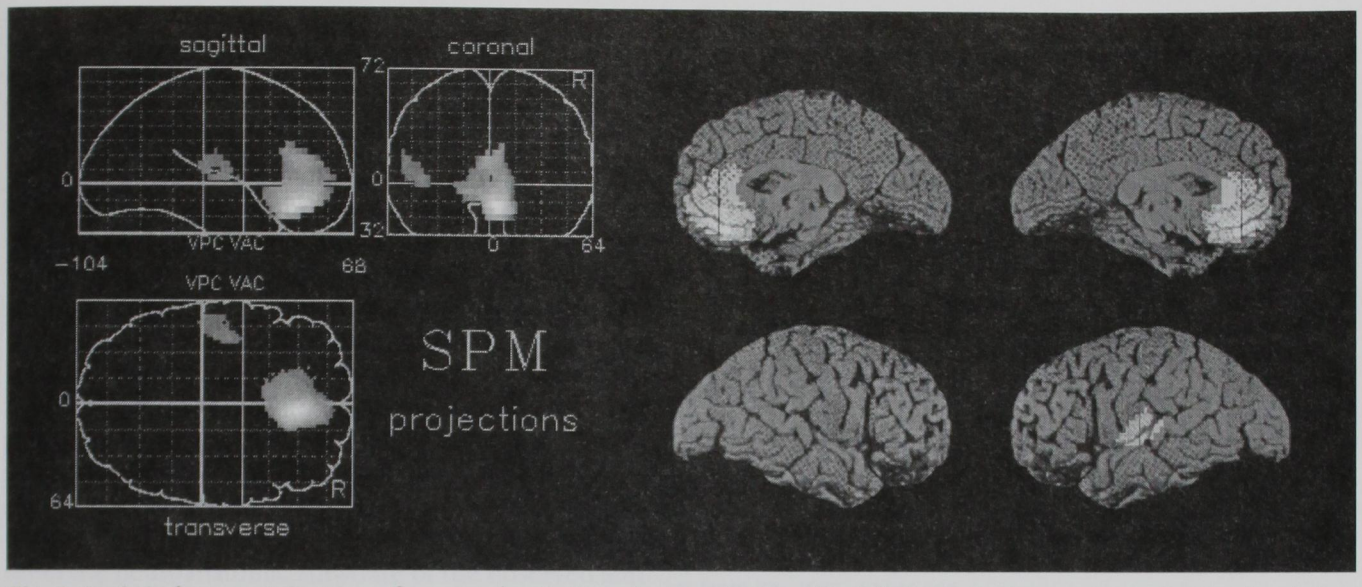

Fig. 3. Statistical parametric maps of neuronal activation during nitrous oxide inhalation in the presence of pain stimulation ([PS + Nitrous Oxide] − [PS]). The activation pattern reflects pain-modulated nitrous oxide responses because only activations associated with pain alone ([PS]) are subtracted from the image obtained during the combined administration of pain and nitrous oxide ([PS + Nitrous Oxide]). Regional brain activation is represented as described in figure 1, and the relevant anatomic assignments are specified in detail in table 6. See figure 1 for explanations of views and abbreviations.

in different areas than those associated with pain (table 4).

The likelihood that the pain activation pattern observed during nitrous oxide inhalation is related to this agent's antinociceptive effect is supported by the association with a decreased subjective pain experience, as reflected by the significantly lower visual analog scale scores. Limitations in this interpretation are posed by effects of nitrous oxide other than analgesia and the nonrandom order of PET scans. Although they were not measured, subjective effects of nitrous oxide, such as mild sedation and euphoria reported by each volunteer, could result in cerebral responses that interfered with the pain-related activation during nitrous oxide inhalation, thus contributing to the observed activation pattern. Participants were blinded to whether room air or nitrous oxide was being administered, but because room air conditions (*e.g.*, [Control], [PS]) always preceded nitrous oxide conditions ([Nitrous Oxide], [PS

Table 6. Areas of rCBF Increases during Nitrous Oxide Inhalation in the Presence of Pain ([PS + Nitrous Oxide] − [PS])

Region	Brodmann's Area	Coordinates			Percentage rCBF Increase	z-Score
		x	y	z		
Infralimbic area (R)	25	4	32	−16	4.2	4.402
Orbitofrontal cortex (R)	11	4	34	−12	4.9	4.498
Orbitofrontal cortex (R)	10	8	44	−8	5.0	3.783
Anterior cingulate (L)	24	− 8	28	0	4.2	2.896
Anterior cingulate (R)	24	8	32	0	5.0	3.368
Anterior cingulate (L)	24	−10	42	4	4.5	2.420
Anterior cingulate (R)	24	6	40	4	3.1	3.196
Anterior cingulate (L)	24	−2	34	12	3.6	3.170
Anterior cingulate (R)	24	2	28	20	3.8	2.775

Only areas with a z-score > 2.326 ($P < 0.01$) are listed. Coordinates as in table 3.

PS = pain stimulation; R = right; L = left.

Table 7. Comparisons of Percentage rCBF Changes Obtained by Point Analysis, in Areas of Occipital Cortex Activated by Visual Stimulation during Nitrous Oxide *versus* Room Air Inhalation ([VS], [VS + Nitrous Oxide])

Region	Brodmann's Area	Coordinates			% rCBF Change [VS]	% rCBF Change [VS + Nitrous Oxide]
		x	y	z		
Gyrus occipitalis inferior (L)	17	−12	−90	−8	6.3 ± 1.56	7.4 ± 2.12
Gyrus occipitalis inferior (R)	17	20	−92	−8	6.5 ± 2.71	8.9 ± 1.70
Gyrus occipitalis inferior (L)	18	−24	−90	−4	5.8 ± 0.82	5.9 ± 1.85
Gyrus occipitalis inferior (R)	18	18	−88	−4	5.9 ± 1.60	7.2 ± 0.96
Cuneus (L)	17	−8	−96	4	2.6 ± 0.90	2.3 ± 1.56
Cuneus (R)	17	4	−94	4	2.7 ± 1.10	2.4 ± 1.69
Cuneus (L)	18	−18	−88	4	3.7 ± 1.37	4.7 ± 1.14
Cuneus (R)	18	22	−90	4	2.1 ± 1.13	2.9 ± 1.60
Cuneus (L)	18	−18	−92	8	3.8 ± 1.52	3.3 ± 1.66
Cuneus (R)	18	12	−92	8	2.1 ± 1.85	2.5 ± 1.69

The magnitude of visual activation did not show significant difference between [VS] and [VS + Nitrous Oxide] conditions ($P > 0.05$). Coordinates as in table 3. VS = visual stimulation; L = left; R = right.

+ Nitrous Oxide]), the possibility of some order effect cannot be ruled out.

Although nitrous oxide inhalation significantly decreased the subjective pain experience, the stimulus was still rated as painful. The contradiction that complete elimination of activation in the thalamus, anterior cingulate, and supplementary motor area is associated only with partial pain relief may be explained by the possibility that, during nitrous oxide inhalation, pain-related net metabolic responses only decreased to a level undetectable by PET. Alternatively, nociceptive processes that involve parallel activation of both excitatory and inhibitory neurons, resulting in minimal net changes in rCMR, could be responsible for the significantly decreased but still present pain perception.

In principle, use of rCBF as an indicator of regional neuronal activity could be confounded in several ways. Any observed rCBF changes could be due either to nitrous oxide-induced alterations in arterial carbon dioxide content[25] or to rCBF/$rCMR_{glu}$ uncoupling.[12] Another potential confounding variable could be nitrous oxide's effects on the responsiveness of the cerebral vasculature.[16] The first possibility is precluded by the lack of change detected by capnography. The second possibility was eliminated with our recent finding that rCBF/$rCMR_{glu}$ coupling is maintained during 20% nitrous oxide inhalation, using the ^{18}F-deoxyglucose PET technique to compare nitrous oxide-induced changes in rCBF with changes in regional cerebral glucose metabolic rate.[11]

To control for the possibility that nitrous oxide directly affects cerebral vasculature responsiveness independent of neuronal activation, we compared occipital rCBF increases evoked by visual stimulation in the presence and absence of nitrous oxide. The response characteristics of visual stimulation are well described under physiologic circumstances showing an approximately 40% rCBF increase in the occipital cortex,[17] therefore providing a large and sensitive signal. Because 20% nitrous oxide has been shown not to perturb the metabolic activity of the primary visual cortex,[11] significant differences between the magnitude of visual activation in the presence and absence of nitrous oxide would have to be attributed to nitrous oxide-related perturbation of cerebral vascular reactivity. There were no significant differences in the visual activation regardless of whether nitrous oxide was present, even with the relaxed criteria for significance of $P < 0.05$. This indicates that rCBF reactivity remains intact during exposure to 20% nitrous oxide, thus permitting us to interpret our pain - nitrous oxide interaction data directly. The effects of higher concentrations, however, on vascular reactivity in this paradigm are not known.

Careful analysis of our findings in light of known neuroanatomic and electrophysiologic data can be used to map the sites of nitrous oxide's analgesic action. Nitrous oxide could suppress medial thalamic neuronal activity directly, by influencing intrathalamic circuits, or indirectly, by affecting thalamic afferent mechanisms. Electrophysiologic studies indicate that nitrous oxide increases the firing of thalamic reticular inhibitory neurons, thereby increasing the inhibitory influence on thalamic somatosensory relay nuclei and consequently decreasing net corticopetal transmission.[26] Alterna-

tively, the depression of thalamic activation could be the result of nitrous oxide's modulatory effect on ascending pain pathways, or other thalamic afferents.

Because infralimbic and orbitofrontal activation occurs exclusively during pain stimulation in the presence of nitrous oxide ([PS + Nitrous Oxide]), and this activation pattern is associated with significantly decreased pain perception, it is likely that these areas contribute to the antinociceptive effect of nitrous oxide. Infralimbic and orbitofrontal stimulation have been shown to produce analgesia,[27] possibly through their connections with ascending and descending pain-modulatory pathways.[28] Another possibility is that bilateral anterior cingulate activation, produced by nitrous oxide both in the absence ([Nitrous Oxide]) and presence of pain stimulation ([PS + Nitrous Oxide]), alone or together with the infralimbic and orbitofrontal responses, represents the neuroanatomic substrate of the observed antinociceptive effect.

Comparing our results with human PET findings addressing the cerebral sites of opioid analgesia may reveal antinociceptive mechanisms common to systemic analgesics. The available data suggest that the primary loci of action are in the thalamus and in the cortical projections of the medial pain system. Jones *et al.*[7] found activation of discrete areas of the anterior cingulate and prefrontal cortices when morphine was administered systemically in one patient with chronic pain. However, only activation associated with morphine analgesia was reported; no data were presented on the effects of pain or morphine alone, which would allow a more complete assessment of pain-opioid interactions. We have reported anterior cingulate activation, and bilateral thalamic deactivation, after fentanyl systemically administered during a peripheral pain stimulus.[8] The thalamus seems to be a common target for both fentanyl and nitrous oxide. But because fentanyl is associated with neither activation nor depression in the anterior cingulate cortex, nor activation in the infralimbic and orbitofrontal areas when administered in the presence of pain, these two analgesics appear to modulate pain perception *via* different neural circuits.

In summary, evidence is presented that nitrous oxide's antinociceptive effects in humans are associated with the abolition of pain-evoked activation in the thalamus, area 24 of the anterior cingulate cortex, and supplementary motor area, as well as activation of the infralimbic and orbitofrontal cortices. Responses in the latter areas raise the possibility that nitrous oxide activates ascending and descending antinociceptive modulatory pathways in addition to depressing pain-induced cerebral responses. These data provide evidence that analgesic concentrations of nitrous oxide modulate cerebral pain processing, at least in part, through the medial pain system.

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