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ORIGINAL ARTICLE

Elevated brain lactate responses to neural activation in panic disorder: a dynamic 1H-MRS study

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Converging evidence suggests that patients with panic disorder have a metabolic disturbance that may influence the regulation of arousal systems and confer vulnerability to 'spontaneous' panic attacks. The consistent finding of elevated brain lactate responses to various metabolic challenges in panic disorder appears to support this model, although the mechanism of this effect is not understood. Several mechanisms have been proposed to account for elevated brain lactate responses in panic disorder, including (1) brain hypoxia due to excessive cerebral vasoconstriction, and (2) a metabolic disturbance affecting lactate metabolism. Recent studies have shown that neural activation (for example, sensory stimulation) causes local lactate accumulation in the presence of increased oxygen availability. The current study used proton magnetic resonance spectroscopic measures of visual cortex lactate changes during visual stimulation in 15 untreated patients with panic disorder and 15 matched volunteers to critically test these two proposed mechanisms of elevated brain lactate responses in panic disorder. Visual cortex lactate/*N*-acetylaspartate increased during visual stimulation in both groups. The increase was significantly greater in the panic patients than in the comparison group. There were no group differences in end-tidal pCO_2. The finding that visual stimulation leads to significantly greater visual cortex lactate responses in panic patients is not predicted by the hypoxia model. These results suggest that a metabolic disturbance affecting the production or clearance of lactate is the cause of the elevated brain lactate responses consistently observed in panic disorder and provide further support for metabolic models of vulnerability to this illness.

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Introduction

Panic disorder (PD) is a potentially disabling condition affecting 1.5–3.5% of the population.[1,2] Although psychosocial factors are very important in this condition, family and twin studies demonstrate that PD has a significant heritable component.[3,4] However, the neurobiological mechanisms of vulnerability to this disorder are not understood. Spontaneous panic attacks, the defining feature of PD, can be characterized as dysregulated paroxysms of arousal associated with a sense of imminent incapacitation. The central regulation of arousal is influenced by many of the same chemoreceptive mechanisms involved in the regulation of respiration. These chemoreceptive mechanisms are highly sensitive to variations in pH and pCO_2 and also respond to variations in other metabolic parameters.[5–8] Converging evidence supports the hypothesis that patients with PD have an underlying metabolic abnormality. PD patients are unusually susceptible to experiencing panic attacks in response to various metabolic challenges, including sodium lactate infusions,[9,10] respiratory stimulation (via carbon dioxide inhalation[11] or doxapram injection[12]) and caffeine ingestion.[13] PD patients also have exaggerated endogenous lactate responses to a variety of metabolic challenges[14–17] and demonstrate abnormal respiratory patterns.[18,19] Both abnormal respiratory patterns and exaggerated endogenous lactate responses persist after clinical remission, suggesting they reflect a trait feature of patients with PD.[16,19]

Understanding the mechanism of exaggerated brain lactate responses to metabolic challenges in PD may provide important clues about an underlying metabolic disturbance in this disorder. Several possible mechanisms of these responses have been proposed, including (1) brain hypoxia resulting from excessive cerebral vasoconstriction;[16,20,21] and (2) a metabolic disturbance affecting lactate metabolism.[14] The current study tests the predictions of these two contrasting models.

Many investigators, using different metabolic challenges, have observed abnormal endogenous

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lactate responses in patients with PD. Exaggerated increases in serum lactate following both exercise and caffeine ingestion have been reported in PD.[13,15,22,23] However, the exercise and caffeine findings may have been confounded by a lower level of aerobic fitness and/or a lesser degree of pharmacological tolerance to caffeine, respectively, in the panic patients compared to the control subjects.[24] Controlled hyperventilation has been a useful paradigm for studying lactate metabolism in PD. Hyperventilation causes an intracellular alkalosis, which leads to increased production of lactic acid as a homeostatic response in defense of intracellular pH. Panic patients demonstrate significantly greater increases in serum lactate during and after hyperventilation than control subjects, in spite of similar end-tidal pCO_2 levels.[14,17,25] Studies of lactate infusions have shown similar evidence for increased lactate responses in PD. Sodium lactate infusion produces a significant metabolic alkalosis and paradoxically, a secondary respiratory alkalosis.[9] The latter reflects lactate's action as a respiratory stimulant.[26,27] Thus, sodium lactate infusion, like hyperventilation, leads to increased production of endogenous lactic acid as a homeostatic response to alkalosis. Of the many published lactate infusion studies, only two examined patient versus control subject comparisons of serum lactate levels matched for total volume of exogenous lactate infused. Both found evidence for a greater increase in lactate in the panic patients.[9,10] More recent studies have used proton magnetic resonance spectroscopy (1H-MRS) to examine lactate responses within the brain in PD patients. Three studies of sodium lactate infusions and one study of hyperventilation have all observed significantly greater increases in brain lactate in PD patients.[16,28–30] Some investigators proposed that the abnormal lactate response resulted from a disturbance in the dynamics of lactate metabolism in PD,[14] while others attributed it to cerebral hypoxia due to an exaggerated cerebral vasoconstriction response to alkalosis in PD.[16,20,21]

It is well known that lactate accumulates under hypoxic conditions. During hypoxia, ATP can be produced directly from the metabolism of glucose to lactate without any requirement for oxygen (anaerobic glycolysis). The most common cause of hypoxic lactate accumulation is a failure of either respiration or tissue perfusion to keep pace with metabolic demand for oxygen, leading to reliance on this anaerobic pathway for energy production. Alkalosis leads to vasoconstriction in the cerebral vasculature.[31] It is possible that alkalosis-induced cerebral vasoconstriction could progress to the point of hypoxia, although brain hypoxia is not observed in healthy animals or humans except at extraordinarily high pH levels.[17] However, two studies report a significant exaggeration of the normal cerebral vasoconstriction response to alkalosis in PD patients.[32,33] The hypoxia model proposes that exaggerated vasoconstriction progressing to the point of hypoxia is responsible for the exaggerated brain lactate responses to alkalotic challenges in PD. In contrast, the metabolic model begins with recognition that lactate normally increases during alkalosis under fully aerobic conditions as part of a homeostatic mechanism to restore normal pH, and proposes that a disturbance affecting the dynamics of lactate metabolism is responsible for the exaggerated lactate response to alkalosis in PD.

It was long believed that significant lactate accumulation occurred only under hypoxic conditions (a view that still appears in some medical textbooks[34]). However, it is now known that lactate accumulation occurs regularly under aerobic conditions in the brain and other tissues and that lactate is an important metabolic fuel.[35,36] Although its exact role as a metabolic fuel is not yet fully understood, studies have shown that lactate is the end product of glycogenolysis in astroglia,[37,38] that accumulation of lactate in brain parenchyma is a normal occurrence during neural activation under fully aerobic conditions[39,40] and that lactate is a significant substrate for neuronal oxidative metabolism.[36,41] The hypoxia model of PD interprets the exaggerated brain lactate responses to alkalosis as confirmation that alkalosis-induced vasoconstriction has caused hypoxia in the PD patients. The inference that elevated brain lactate responses observed in PD are due to hypoxia is critical to this model, as this is the only experimental evidence that episodes of brain hypoxia occur in PD. However, the assumption that brain lactate accumulation signifies brain hypoxia in PD is inconsistent with current knowledge of the role of lactate in brain metabolism.[36,41] The current study tests this idea experimentally, by examining whether elevated brain lactate responses occur in PD patients when hypoxia can be excluded as a mechanism. If elevated brain lactate responses in PD do not signify hypoxia, then there is no evidence that episodes of brain hypoxia occur in PD.

In keeping with its role as a metabolic fuel, recent 1H-MRS studies have demonstrated consistent increases in visual cortex lactate concentration during visual stimulation in human subjects.[40,42,43] Visual stimulation, like other methods of neural activation, also leads to an increase in local brain blood flow and oxygen availability. This activation-induced increase in blood flow and oxygen availability is one of the most well-established observations in neuroscience and is the physiological basis of most modern functional neuroimaging techniques.[44,45] Thus, 1H-MRS measures of brain lactate during visual stimulation offer an opportunity to test contrasting predictions of the 'hypoxia' and 'metabolism' models of exaggerated lactate responses in PD. PD patients are known to have episodes of elevated brain lactate, which could be caused by hypoxia or by a disturbance of lactate metabolism. Since local brain hypoxia does not occur during sensory stimulation, the 'hypoxia' model predicts that the characteristically exaggerated lactate responses of PD patients will not be evident under these conditions. Since the 'metabolism' model posits a disturbance affecting the dynamics of lactate

metabolism, this model predicts that lactate responses will be exaggerated whenever lactate metabolism is stimulated. *In vivo* studies in both animals and humans have shown that neural activation is accompanied by increased glycolysis under fully aerobic conditions (aerobic glycolysis) and results in an increase in local brain lactate concentration.[39,40,42,43,45] Thus, the 'metabolism' model predicts that the characteristically exaggerated lactate responses of PD patients will be observed in the visual cortex during visual stimulation. We used 1H-MRS measures of visual cortex lactate responses to visual stimulation in PD patients and healthy comparisons subjects to test the contrasting predictions of these two models.

Materials and methods

Subjects

Seventeen untreated PD patients (aged 21–55 years) and 16 healthy volunteers matched for gender, age, education and caffeine consumption were recruited by newspaper advertisement. Usual daily caffeine consumption was estimated with a structured interview about dietary practices, using values for caffeine content in food products suggested by Barone and Roberts.[46] This study was approved by the IRB at the University of California Davis. After complete description of the study, subjects gave written informed consent. A psychiatrist (RJM) evaluated all subjects using the Structured Clinical Interview for DSM-IV.[47] All patients had a current primary diagnosis of PD (14 with agoraphobia), two had secondary generalized anxiety disorder, two had secondary social phobia and one had a past episode of major depression. No other lifetime DSM-IV diagnoses were present in any patients or control subjects, except for two control subjects with past alcohol abuse in remission for more than 15 years. All subjects were free of other illnesses and medications affecting brain, vascular or respiratory function. Eight patients had previously taken serotonin reuptake inhibitors (discontinued $\geqslant 4$ weeks before scanning, median = 1 year before scanning). Two patients reported recent, infrequent low-dose benzodiazepine use (last use 2 and 11 days before scanning).

Experimental procedures

All subjects underwent habituation to a 'mock' scanner prior to entering the MRI scanner. Anxiety levels and end-tidal pCO_2 were monitored during the habituation period, which lasted from 3 to 30 min, depending on the subject's comfort. All subjects eventually tolerated the mock scanner well. Following habituation, subjects entered the MRI scanner for two brief anatomical scans. Then, continuous 1H-MRS data were collected for 15.6 min. During the first 5 min, subjects were instructed to relax with eyes closed. During the subsequent 10.6 min, subjects were instructed to observe a black and white radial checkerboard undergoing 8 Hz pattern reversal flicker projected to a screen at their feet. Subjects were instructed to press a button whenever the checkerboard changed briefly from radial to rectangular and back to radial, which occurred approximately 4 times per minute. This embedded vigilance task was intended to engage and monitor the subject's attention to the visual stimulus. In 14 patients and 14 control subjects, 1H-MRS data were collected for an additional 12 min immediately following the offset of the visual stimulus. Subjects were instructed to relax with eyes closed during this time period. Anxiety was rated with the Acute Panic Inventory (API)[48] and the State-Trait Anxiety Inventory-State version (STAI-S)[49] immediately before scanning and post-scanning retrospectively for the period of visual stimulation. To monitor for potential pH changes due to hyperventilation, end-tidal pCO_2 was recorded and quantified throughout scanning using a face mask connected to an infrared capnometer, as previously described.[14] The clinician-rated Panic and Agoraphobia Symptom Scale (PAS) and the patient-rated Anxiety Sensitivity Index were administered to all patients prior to scanning.[50,51]

MRI methods

Data were acquired with a 1.5 T MRI system (Signa Horizon NV/i; GE Medical Systems, Milwaukee, WI, USA) using a three-inch surface coil positioned under the occiput. Sagittal T1 and axial T2 FSE scans were acquired for localization. A single-voxel 1H-MRS scan was acquired using a PRESS sequence as follows: pulse sequence database, probe-p; echo time (TE), 288 ms; repetition time, 1500 ms; phase cycling, eight; acquisitions, 624 (for baseline and visual stimulation) and 480 (for the post-stimulation recovery period); bandwidth, 2500 Hz. These parameters generated frames of spectral data with a temporal resolution of 12 s. As in prior studies demonstrating visually evoked lactate responses, a TE of 288 ms was used to minimize lipid and macromolecule signal overlapping lactate.[40,42,43] Visual stimulation is expected to cause neural activation and lactate accumulation in primary visual cortex. Thus, data were acquired in the axial plane from an 18.75 cc voxel centered on bilateral calcarine fissures. A localization gradient order of S/I, A/P, R/L was used to further minimize lipid artifact originating outside the voxel.[43,52]

Data analysis

We previously observed a delay of 36 s (three frames) following the onset of visual stimulation before an increase in lactate was detected.[43] Thus, we defined two time periods for the primary analysis: 'baseline' (initial 5 min with eyes closed) and 'visual stimulation' (the 10 min of visual stimulation subsequent to the initial 36 s of stimulation). MRUI software[53] was used to zero-fill and phase-align the individual frames of MRS data, which were then summed across the time period of interest and apodized with a 4 Hz Gaussian function in the time domain. Using custom software,[43] the *N*-acetylaspartate (NAA) peak

frequency was set to 2.01 p.p.m. and the lactate/NAA ratio was quantified by automated peak integration (lactate = sum from 1.20 to 1.43 p.p.m., NAA = sum from 1.87 to 2.15 p.p.m.). As in prior studies of visual stimulation,[40,42,43] lactate was quantified relative to NAA, rather than creatine (Cr). Lactate increases are expected to result primarily from neuronal activation. Since NAA provides a surrogate marker for neuronal integrity, NAA values serve to normalize lactate to the volume of healthy neuronal tissue within the voxel. Total Cr values were also quantified (sum from 2.96 to 3.10 p.p.m.). The percent change in lactate/NAA from baseline was quantified for five consecutive 2 min intervals during visual stimulation and analyzed with repeated measures analysis of variance (rANOVA). The prediction of a greater increase in lactate/NAA during visual stimulation in PD was tested by a main effect for group ($\alpha = 0.05$). Confirmation of this prediction would be evidence against the hypoxia model. Failure to confirm this prediction would be evidence against the metabolic model. Similarly, the percent change from baseline end-tidal pCO_2 over five consecutive 2 min intervals during visual stimulation was also analyzed by rANOVA. A second analysis was conducted to examine lactate levels during the post-stimulation recovery period. For this analysis, the percent change in lactate/NAA from baseline was quantified for two 6 min 'recovery' periods and analyzed with rANOVA. The prediction of a more persistent elevation of lactate/NAA during the post-stimulation recovery period in PD was tested by a main effect for group ($\alpha = 0.05$). Other group comparisons were made by unpaired *t*-test.

Limitations and validation

The values obtained for NAA, Cr and lactate using the peak integration method include some signal from overlapping metabolites, macromolecules and lipids, although the latter two are minimized by the long TE. Furthermore, both the long TE and partial volume effects make accurate estimation of absolute metabolite concentrations difficult. However, these limitations are mitigated by the fact that the hypothesis being tested relies only on relative changes in lactate over time within each subject, and lactate is the only metabolite for which the concentration is expected to change in response to the visual stimulus.

Subsequent to collection of the data presented here, we implemented a lactate J-editing pulse sequence that effectively separates lactate from potentially overlapping lipid and macromolecule signals.[54] In two groups of healthy volunteers, we measured the lactate response to visual stimulation using the new J-editing sequence ($N = 5$) and the long TE PRESS sequence used for this study of PD patients ($N = 6$). The results of the two acquisition methods were very similar, except that larger effect sizes for the lactate increase during visual stimulation were observed with the J-editing sequence. Based on spectra acquired with the J-editing sequence, we calculated that the relative magnitude of lipid and macromolecule signal overlapping lactate at TE = 144 was 17% of the magnitude of the edited lactate signal. As T2 is much longer for lactate than lipid or macromolecules, this proportion is expected to be even smaller at TE = 288. These methods and results, which support the validity of the methods used in this study of PD patients, are described in detail in the Supplementary information.

Results

No subject reported a panic attack during collection of 1H-MRS data. One patient experienced a panic attack at the beginning of the scanning session. She was removed from the scanner, but voluntarily returned to complete data collection 1 h later without recurrence of panic symptoms. Two patients and one control subject had inadequate lipid suppression (visible lipid signal overlapping the lactate doublet). These subjects were excluded from all analyses, resulting in sample sizes of 15 PD patients and 15 control subjects. The lactate doublet centered at 1.32 p.p.m. was visible in all included subjects (Figure 1). Patients and control subjects performed similarly on the vigilance task embedded in the visual checkerboard stimulus (mean hit rate ($\pm$ s.d.) = 99% ($\pm$ 1) and 98% ($\pm$ 4), respectively, $t = 0.4$, d.f. = 24, NS (data was lost in four subjects due to technical errors)).

Demographic, rating scale and baseline measures are shown in Table 1. Patients had higher prescan state anxiety ratings than control subjects, but did not differ from control subjects on baseline values of lactate/NAA, any other 1H-MRS measures or end-tidal pCO_2. The change in symptom ratings from prescan to visual stimulation did not differ

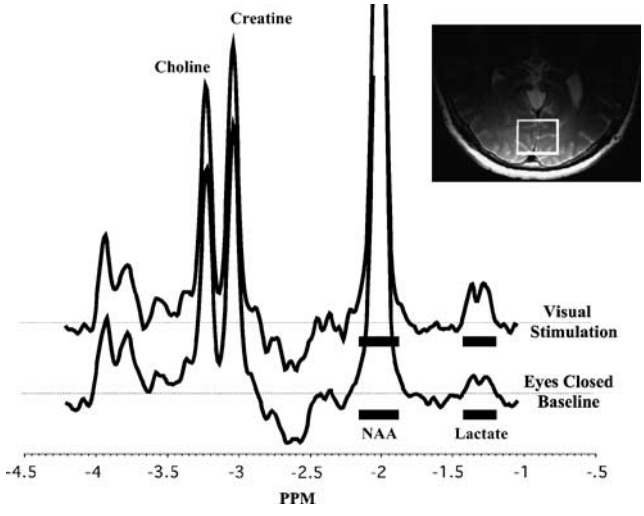

Figure 1 Summed spectra for baseline and visual stimulation conditions in one patient illustrating increased lactate signal during visual stimulation. The integration limits for lactate and *N*-acetylaspartate (NAA) are shown (solid bars). Spectra amplitudes are normalized to the height of the NAA peak. Inset shows voxel location over calcarine fissures in the axial plane.

Table 1 Demographic data and baseline values

	PD	*Control*	*Unpaired* t-*test*
N	15	15	
Female (%)	67%	67%	
Age (years)	37.5 (9.2)	37.1 (6.9)	$t=0.2$, NS
Education (years)	15.3 (1.8)	15.9 (1.0)	$t=1.2$, NS
Usual daily caffeine (mg day^{-1})	57.8 (63)	73.4 (89)	$t=0.5$, NS
Prescan STAI state score	41.3 (10.6)	25.4 (9.8)	$t=4.3$, $P<0.001$
Prescan API score	2.1 (2.7)	0.3 (1.0)	$t=2.4$, $P<0.025$
Baseline respiratory rate (b.p.m.)	13.7 (4.2)	15.1 (4.2)	$t=0.9$, NS
Baseline end-tidal pCO_2 (mm Hg)	40.6 (4.4)	40.9 (3.9)	$t=0.2$, NS
Baseline NAA (arbitrary units)	95.7 (13.9)	104.3 (22.5)	$t=1.3$, NS
Baseline creatine (arbitrary units)	30.6 (3.9)	32.4 (6.2)	$t=1.0$, NS
Baseline NAA/creatine ratio	3.13 (0.3)	3.22 (0.3)	$t=0.9$, NS
Baseline lactate/NAA (%)	6.54% (0.9)	6.35% (0.8)	$t=0.6$, NS
Baseline lactate/creatine (%)	20.5% (2.6)	20.5% (3.1)	$t=0.1$, NS

Abbreviations: API, Acute Panic Inventory; NAA, *N*-acetylaspartate; NS, nonsignificant; PD, panic disorder; STAI, State-Trait Anxiety Inventory-State version.
Prescan ratings were obtained on the scanner bed immediately prior to scanning. Baseline values were obtained during a 5 min scanning period of resting with eyes closed. MRS data were obtained from a voxel placed bilaterally in the primary visual cortex. For respiratory measures, d.f. = 25 (two patients and one control subject were unable to wear the face mask). For all other measures, d.f. = 28.

significantly between groups for either the API (PD = $+2.5 \pm 4.8$, control = 0.0 ± 1.0; $t=1.9$, d.f. = 28, NS) or the STAI-S (PD = $+0.5 \pm 7.8$, control = -1.3 ± 7.4; $t=0.6$, d.f. = 28, NS). Patients reported a mean of 3.3 (± 2.2) panic attacks (by DSM-IV criteria) and had a mean score on the PAS of 22.7 (± 10.5) during the week preceding scanning. Patients had a mean score of 36.9 (± 14) on the ASI.

Repeated measures analysis of variance of percent change from baseline lactate/NAA during the period of visual stimulation showed a significant main effect of group ($F=5.21$, d.f. = 1, 28, $P=0.030$), with PD patients having significantly greater increases in visual cortex lactate/NAA (effect size (Cohen's d) = 0.83) (Figure 2). Neither the main effect of time ($F=0.80$, d.f. = 4, 112) nor the interaction of group and time ($F=0.74$, d.f. = 4, 112) was significant. Both the patient and control groups showed a significant increase in lactate/NAA over the baseline value during the first 2-min period of visual stimulation by paired t-test (PD: $t=3.5$, d.f. = 14, $P=0.003$; control: $t=2.2$, d.f. = 14, $P=0.045$). The patient group showed significant elevations of lactate/NAA over baseline values during the second, third and fifth 2-min periods (all $t>3.1$, d.f. = 14, $P<0.01$). Analyzing absolute change in lactate/NAA gave the same pattern of results as percent change in lactate/NAA. Normalizing lactate values to total Cr rather than NAA produced the same pattern of results (Table 2). No significant effects were observed for NAA/Cr. A significant main effect for time was observed for end-tidal pCO_2, with progressively decreasing values during visual stimulation. There was no group effect or group by time interaction for pCO_2 (Table 2).

Analysis of lactate/NAA values during the 12 min post-stimulation recovery period showed a significantly greater percent change increase over baseline values in the panic patients than control subjects (PD: $+8.1 \pm 14.2$, control: -2.9 ± 7.7; $t=2.5$, d.f. = 26, $P=0.017$). Figure 3 shows mean lactate/NAA values as percent change from baseline during visual stimulation and recovery in the two groups. Effect size (Cohen's d) for the group difference across the visual stimulation and recovery periods = 1.08. End-tidal pCO_2 did not differ between patients and control subjects during the recovery period ($t=0.2$, d.f. = 24, NS).

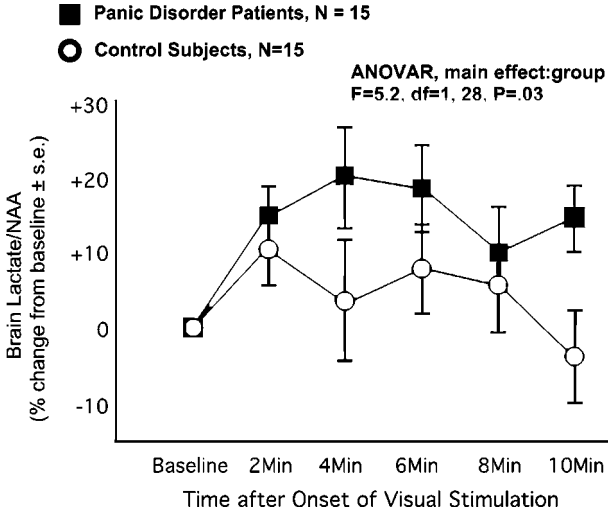

Figure 2 Percent change from baseline in visual cortex lactate/*N*-acetylaspartate (NAA) quantified over five consecutive 2 min periods of visual stimulation. Over all 10 min of visual stimulation, lactate/NAA increase was significantly greater in 15 panic disorder patients compared to 15 control subjects.

Table 2 Changes from baseline in other metabolite ratios and end-tidal pCO_2 during visual stimulation

	Minutes of visual stimulation						*F*	*d.f.*	P
	1–2	*3–4*	*5–6*	*7–8*	*9–10*				
Lac/Cr						G	4.93	1, 28	0.035
PD	+15.9 (18)	+21.8 (26)	+19.5 (22)	+9.7 (26)	+17.1 (20)	T	0.71	4, 28	NS
Control	+11.1 (19)	+4.9 (32)	+7.6 (24)	+5.1 (25)	−2.4 (25)	GxT	0.73	4, 112	NS
NAA/Cr						G	2.02	1, 28	NS
PD	−0.1 (3.3)	+1.8 (5.2)	+0.5 (5.6)	+0.6 (3.5)	+1.9 (5.1)	T	1.16	4, 28	NS
Control	−1.0 (3.1)	−0.2 (3.7)	−0.9 (3.7)	−0.7 (4.2)	0 (2.9)	GxT	0.13	4, 112	NS
pCO_2						G	1.06	1, 25	NS
PD	+0.6 (15)	−1.3 (13)	+1.6 (13)	+0.2 (16)	−3.1 (16)	T	2.80	4, 25	0.030
Control	−1.6 (12)	−3.3 (11)	−4.9 (10)	−6.5 (13)	−9.8 (16)	GxT	0.84	4, 100	NS

Abbreviations: Cr, total creatine; G, main effect for group; GxT, interaction effect; Lac, lactate; NAA, *N*-acetylaspartate; NS, nonsignificant; PD, panic disorder; T, main effect for time.
Tabled values at each time interval are mean (s.d.) % change from baseline. Results of repeated measures ANOVA are shown.

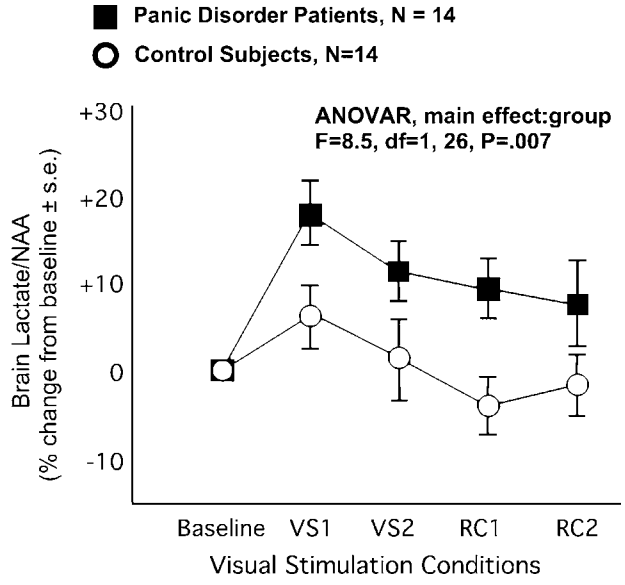

Figure 3 Percent change from baseline visual cortex lactate/*N*-acetylaspartate (NAA) quantified over two consecutive 5 min periods of visual stimulations (VS1 and VS2) followed by two consecutive 6 min periods of recovery with eyes closed (RC1 and RC2). Over all visual stimulation and recovery periods, lactate/NAA increase was significantly greater in 14 panic disorder patients compared to 14 control subjects.

Discussion

In agreement with prior 1H-MRS studies of visual stimulation in healthy volunteers,[40,42,43] the current study demonstrated a significant increase in visual cortex lactate during visual stimulation in both PD patients and healthy comparison subjects. In addition, we found significantly greater elevations in brain lactate during and following visual stimulation in the PD patients. This confirms and extends the findings of prior studies showing elevated brain lactate responses during metabolic challenges in patients with PD.[16,28–30]

Increased local oxygen availability, rather than hypoxia, in response to stimulus-induced neural activation is one of the most well-established observations in neuroscience.[44,45] Since the hypoxia model attributes the consistently elevated brain lactate responses observed in PD to ischemic hypoxia,[16,20,21] it predicts that the characteristic elevation of brain lactate would not be observed in the visual cortex in PD patients during visual stimulation. The current results contradict the prediction of this model and call into question the inference that episodes of brain hypoxia occur in PD patients.[20] Although this does not disprove the hypoxia model, it substantially weakens the argument that the characteristically elevated brain lactate responses in PD patients are evidence of episodes of brain hypoxia. It should be noted that there is some evidence that a slight, transient decrease in local pO_2 may occur during the first few hundred milliseconds of neural activation.[39] However, if this occurs, it would be quickly supplanted by a longer lasting hyperemia and increase in oxygen availability.[44,45] Thus, even if a transient hypoxia occurs at the beginning of visual stimulation, it cannot be the cause of the sustained elevation of lactate observed in the PD patients.

The metabolic model attributes elevated brain lactate responses during metabolic challenges in PD to a disturbance affecting the dynamics of lactate metabolism.[14] This model predicts that PD patients would show an exaggeration of the normal lactate accumulation in response to visual stimulation. Our findings are consistent with the prediction of the metabolic model. While it remains possible that hypoxia could account for brain lactate accumulation under other circumstances in PD, a more

parsimonious interpretation is that panic patients have a metabolic disturbance affecting the production or clearance of lactate, and that this is responsible for their consistently elevated lactate responses.

PD patients frequently hyperventilate during stressful laboratory situations.[55] Hyperventilation-induced hypocapnia and respiratory alkalosis can directly stimulate the production of lactate via disinhibition of phosphofructokinase, the rate-limiting enzyme of glycolysis.[17,56] However, we found that end-tidal pCO_2 values were eucapnic and did not differ between the PD patients and the control subjects in our study, either at baseline, in response to the visual stimulation, or during the recovery period. Thus, respiratory alkalosis cannot account for the elevated lactate response in the PD group. This agrees with other studies showing elevated lactate responses in PD in the absence of any group differences in end-tidal pCO_2.[14,16,17]

Our findings support the hypothesis that a metabolic disturbance affecting the production and/or clearance of lactate is present in patients with PD. However, this may be an epiphenomenon, neither specific to PD nor an antecedent cause of vulnerability to PD. It may be simply one of many physiological consequences of severe anxiety. If so, the metabolic disturbance should be noted in other disorders characterized by recurrent episodes of severe anxiety, not just PD. In addition, these abnormalities should no longer be observed when PD patients are in clinical remission. Finding that this metabolic disturbance is specific to PD and persists in clinically remitted PD patients would make it a more plausible candidate for a marker of genetic vulnerability to PD. Only one study has examined lactate metabolism in anxiety disorders other than PD. Tancer *et al*.[15] compared untreated PD and social phobia patients and found significantly greater serum lactate responses to caffeine in the PD patients. This suggests a metabolic effect specific to PD. However, the patient groups were not matched for usual caffeine consumption. Three studies have assessed lactate metabolism in small samples of remitted PD patients. Two found that elevated brain lactate responses to a lactate infusion were still present after PD patients were treated and in clinical remission,[28,30] and the third found a significantly elevated brain lactate response to hyperventilation in remitted PD patients.[16] This suggests that abnormal lactate metabolism is not merely a consequence of recent panic attacks. In the current study, PD patients had significantly higher state anxiety than control subjects at the time of scanning. Two *post hoc* analyses were performed to examine the possibility that elevated lactate response in the PD group could be attributed to higher state anxiety. First, group differences in lactate response were unchanged when state anxiety scores were entered as a covariate in the analysis. Second, we created a subgroup of nine PD patients and nine control subjects that did not differ in state anxiety. The lactate response remained significantly elevated in this subgroup of PD patients. These results suggest that state anxiety alone cannot account for the elevated lactate response in the PD group. Studies of subjects at increased risk for PD, but with no prior history of panic attacks, would provide a strong test of the hypothesis that this metabolic disturbance is a marker for vulnerability to PD. Further clinical studies of patients with other anxiety disorders, of PD patients in remission and of individuals at high risk for PD will help determine the potential clinical significance of this metabolic disturbance.

If further studies suggest that a metabolic disturbance affecting the production or clearance of lactate has pathogenic significance in PD, then it will be important to understand the mechanism of this disturbance. Brain lactate production is regulated at several key metabolic steps, including the generation of glucose-6-phosphate (either from glucose or astrocytic glycogen), the activity of phosphofructokinase and the reduction of pyruvate to lactate by lactate dehydrogenase (LDH). These steps can be influenced by several neurotransmitters and neuromodulators, by cyclic AMP, by intracellular pH, by redox status, by energy status and by other metabolic or cellular signaling factors.[17,57,58] Thus, there are numerous potential mechanisms by which lactate production could be increased in PD. Brain lactate is cleared largely by oxidation within mitochondria. A lactate oxidation complex, including MCT1 (a lactate transporter), LDH and cytochrome oxidase, has recently been identified on the mitochondrial inner membrane.[59] Recent studies suggest that neural activation increases glycogenolysis and lactate release by astrocytes.[37,38,60] Neurons readily take up and oxidize lactate, and thus may play a key role in lactate clearance.[61] Reduced neuronal or glial oxidation of lactate within mitochondria could result in elevated brain lactate levels following metabolic challenge in PD. Although most brain lactate is thought to be cleared by mitochondrial oxidation,[59] an unknown fraction is cleared by the cerebrovascular system.[45] A disturbance of cerebrovascular regulation could contribute to elevated brain lactate responses in PD.[32,33]

Lactate is a respiratory stimulant in humans[9] and animals,[26,27] and is a panicogen in PD patients, although the mechanism(s) of these effects are not yet known. Chemosensitive cells in both brainstem and suprapontine regions, including the ventral medulla, locus ceruleus, raphe nuclei, periaqueductal gray, hypothalamus and amygdala, strongly influence the regulation of arousal, as well as respiration and cardiovascular function.[5–8,62,63] Many acid-sensitive chemoreceptors respond selectively to decreases in extracellular fluid (ECF) pH.[6,8] Neuronal activity is often followed by intracellular alkalinization within astrocytes and acidification of brain ECF.[64] The degree of activity-evoked ECF acidification has been correlated with lactate accumulation.[65] Episodes of exaggerated activity-evoked lactate accumulation in chemosensitive nuclei could be integral to the process that triggers the unwarranted arousal reactions, as

well as respiratory and autonomic changes, characteristic of spontaneous panic attacks. Some acid sensing ion channels in peripheral sensory neurons are sensitized by increases in ECF lactate concentration.[66] These peripheral acid sensing ion channels appear to mediate distressing interoceptions associated with lactate accumulation, including anginal pain.[67] These or related mechanisms could mediate an association between elevated lactate responses and 'spontaneous' panic attacks triggered by chemosensitive arousal systems in PD patients.

In addition to its potential effects on chemosensitive arousal systems, lactate is an important substrate for energy metabolism in neurons and glia.[61] If the metabolic disturbance in PD involves reduced mitochondrial oxidative clearance of lactate, it could potentially alter the balance of metabolic pathways regulating neuronal energetics. Recent genetic studies have reported an association between the Bcl-2 gene and anxiety in mice.[68,69] This gene encodes a protein involved in regulating mitochondrial energetics within neurons.[69,70] Other genetic factors associated with glycolytic and oxidative metabolism have been linked to anxiety in humans[71] and mice.[72] If further studies demonstrate an association between trait vulnerability to PD and elevated lactate responses, then understanding the metabolic mechanism of this association will be an important priority.

In summary, untreated patients with PD showed an exaggerated brain lactate response to visual stimulation compared to matched control subjects. This result is consistent with the metabolic model and contrary to the hypoxia model of elevated brain lactate responses in PD. Our findings suggest that PD patients have a metabolic disturbance that results in increased production and/or decreased clearance of lactate in the brain and are consistent with metabolic models of vulnerability to this disorder. Further studies are required to determine the pathophysiological significance and the mechanism of this metabolic disturbance in patients with PD.

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