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Glucose metabolism in the amygdala in depression: Relationship to diagnostic subtype and plasma cortisol levels

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Abstract

In a previous positron emission tomography (PET) study of major depression, we demonstrated that cerebral blood flow was increased in the left amygdala in unipolar depressives with familial pure depressive disease (FPDD) relative to healthy controls [J. Neurosci. 12 (1992) 3628.]. These measures were obtained from relatively low-resolution PET images using a stereotaxic method based upon skull X-ray landmarks. The current experiments aimed to replicate and extend these results using higher-resolution glucose metabolism images and magnetic resonance imaging (MRI)-based region-of-interest (ROI) analysis. The specificity of this finding to FPDD was also investigated by assessing depressed samples with bipolar disorder (BD-D) and depression spectrum disease (DSD). Finally, the relationship between amygdala metabolism and plasma cortisol levels obtained during the scanning procedure was assessed. Glucose metabolism was measured using PET and ^{18}F-fluorodeoxyglucose (^{18}FDG) in healthy control ($n=12$), FPDD ($n=12$), DSD ($n=9$) and BD-D ($n=7$) samples in the amygdala and the adjacent hippocampus. The left amygdala metabolism differed across groups ($P<.001$), being increased in both the FPDD and BD-D groups relative to the control group. The left amygdala metabolism was positively correlated with stressed plasma cortisol levels in both the unipolar ($r=.69$; $P<.005$) and the bipolar depressives ($r=0.68$; $.1<P<.05$). In contrast, neither significant main effects of diagnosis nor significant relationships with plasma cortisol were evident in post hoc analyses of metabolism in the right amygdala or the hippocampus. Preliminary assessment of BD subjects imaged during remission suggested that amygdala metabolism is also elevated in remitted subjects who are not taking mood-stabilizing drugs, but within the normal range in subjects taking mood stabilizers. These data confirm our previous finding that neurophysiological activity is abnormally increased in FPDD, and extend it to BD-D. These abnormalities were not accounted for by spilling in of radioactivity from the adjacent hippocampus. The correlation between left amygdala metabolism and stressed plasma cortisol levels may conceivably reflect either the effect of amygdala activity on corticotropin-releasing hormone (CRH) secretion or an effect of cortisol on amygdala function.

Keywords: Positron emission tomography; Major depression; Bipolar disorder; Amygdala; Cortisol

1. Introduction

The involvement of the amygdala in emotional behavior has generated interest in this structure's function in pathological emotional conditions such as major depression (Drevets, 2001; LeDoux, 1987; McEwen, 1995; Nishijo et al., 1988). In a previous positron emission tomography (PET) study, we demonstrated that cerebral blood flow (CBF) was abnormally increased in the amygdala and anatomically related parts of the prefrontal cortex (PFC) and thalamus in the depressed phase of major depressive disorder (MDD; Drevets et al., 1992). The

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specificity of these data was limited, however, by the low spatial resolution of the PET images obtained in this study [full width at half maximum (FWHM — the measure of spatial resolution in PET images) = 17 mm] and their dependence upon a skull X-ray-based stereotaxic method for locating regions-of-interest (ROI) (Fox et al., 1985). Moreover, this previous PET study (Drevets et al., 1992) addressed the heterogeneity of MDD by selecting the depressed sample according to familial pure depressive disease (FPDD) criteria (primary MDD in an individual with a first degree relative, i.e., parent, sibling, or offspring, who has MDD, but no first degree relatives with mania, alcoholism, or sociopathy; Winokur, 1982), so the generalizability of this finding to other major depressive subtypes remained unclear.

The current study sought to replicate this observation using higher-resolution PET images and magnetic resonance imaging (MRI)-based colocation of PET measures. The resolution and statistical quality of the PET images were improved by acquiring them with a more technologically advanced PET camera and by assessing neurophysiology in terms of glucose metabolism rather than CBF. While changes in glucose utilization are tightly coupled with changes in CBF during physiological activation (Fox et al., 1988), glucose metabolism images are of superior statistical quality because of their higher radioactive count rate per pixel (Raichle, 1987).

In addition, extension of the results to depressed samples meeting criteria for other primary mood disorders was explored by including samples meeting criteria for depression spectrum disease (DSD; primary MDD in an individual with first degree relatives who have alcoholism or sociopathy, but no first degree relatives with mania) and bipolar disorder (BD; "manic–depressive illness"). In addition to assessing subjects in the depressed phase of BD (BD-D), a sample of bipolar subjects was scanned during the remitted phase (BD-R). Our previous study of unipolar depression showed that unmedicated, remitted subjects with FPDD had a nonsignificant trend toward elevated left amygdala CBF relative to healthy controls, so we hypothesized that amygdala hypermetabolism may also extend to *unmedicated* BD-R subjects.

Finally, the neuroendocrine correlates of amygdala hypermetabolism in depression were explored by assessing its relationship to cortisol secretion. The amygdala plays a role in stimulating corticotropin-releasing hormone (CRH) and cortisol secretion during stress via both indirect, disinhibitory connections to the hypothalamic paraventricular nucleus (PVN) and via CRH expressing neurons intrinsic to the amygdala (Beaulieu et al., 1987; Feldman and Conforti, 1981; Feldman et al., 1994; Gray et al., 1989; Herman and Cullinan, 1997; McEwen, 1995; Raisman and Field, 1971). Thus, the excessive central stimulation of hypothalamic–pituitary–adrenal (HPA) axis activity that is evident in major depression may conceivably result from pathologically increased amygdala activity (Aborelius et al., 1999; Banki et al., 1987; Holsboer, 1995; Krishnan et al., 1991; McEwen, 1995; Musselman and Nemeroff, 1993; Nemeroff et al., 1984).

The PET scanning procedure affords a condition under which the relationship between amygdala metabolism and *stress-related* cortisol secretion can be investigated. In the scanning protocol followed herein, the ^{18}F-fluorodeoxyglucose (^{18}FDG) infusion occurred about 1 h following arterial and intravenous cannulation and following 30–40 min of physical restraint on the PET scanning bed via a rigid thermoplastic mask placed over the face, which fixes the subject's head within the scanner gantry. The circulating glucocorticoid concentrations during the period when glucose utilization is measured are thus expected to reflect the elevation of plasma cortisol secretion induced by these physical–psychological stressors, and *not only* the circadian pattern of cortisol secretion.

2. Methods

2.1. Subject selection

Depressed subjects ages 18–59 who met DSM-IV criteria for recurrent MDD or BD were recruited from ongoing family studies and from the clinical services affiliated with Washington University School of Medicine. Subjects provided informed consent, as approved by the Washington University School of Medicine Institutional Review Board. Exclusion criteria included major medical and neurological disorders, treatment with psychotropic or other medications likely to affect CBF or metabolism within the 3 weeks prior to scanning (except in the case of the BD-R sample, since many BD subjects cannot maintain remission without mood-stabilizing treatments), substance abuse within 1 year prior to scanning, substance abuse or other psychiatric disorders prior to the MDD onset, lifetime history of substance dependence and inability to provide informed consent.

The unipolar depressed group met DSM-IV criteria for recurrent MDD (American Psychiatric Association, 1994), and either FPDD or DSD (Winokur, 1982). The BP-D subjects met DSM-IV criteria for BD, and either currently met DSM-IV criteria for a major depressive episode (BD-D) or for remission (BD-R; American Psychiatric Association, 1994). Control subjects met the same exclusion criteria applied to the depressed groups, but had never met criteria for a major psychiatric (Axis I; American Psychiatric Association, 1994) disorder. On the day of scanning, depression severity was rated by the Hamilton Depression Rating Scale (HDRS) scores (21 item; Hamilton, 1960), anxiety severity by the Spielberger State Anxiety Inventory (SSAI; Spielberger et al., 1970) and the frequency of depressive ideation by the Automatic Thoughts Questionnaire (ATQ; Hollon and Kendall, 1980). In the BD subjects, manic symptoms were rated using the Mania Rating Scale of Young et al. (1978).

2.2. Image acquisition

PET scans of glucose utilization were acquired as subjects rested with eyes closed using a Siemens/CTI 953B (31 contiguous slices 3.375 mm thick; in plane resolution = 4.9 mm FWHM), 5–10 mCi of ^{18}FDG (Phelps et al., 1979) and a 72-min dynamic emission scan (Fiorelli et al., 1992). The last 36 min consisted of nine 4-min frames, which were aligned to one another to reduce the effects of movement using AIR (Woods et al., 1993) and summed for analysis into a single image. The images were filtered to a reconstructed resolution of 8 mm FWHM using a 3-D low-pass Butterworth filter. As previously reported (Drevets et al., 1997), the whole brain glucose metabolism measured in these subject samples using arterial blood sampling and adaptations of the Sokoloff method (Phelps et al., 1979) did not differ across the control, bipolar depressed and unipolar depressed groups. To reduce the variability of the regional measures, a linear normalization was applied by dividing regional activity by whole brain activity to negate the effects of nonspecific global fluctuations on local metabolism.

MRI scans were acquired using a Siemens VISION 1.5T scanner and a 3-D MPRAGE sequence ($T_1 = 300$ ms, TR = 9.7, TE = 4, flip angle = 12°, $1 \times 1 \times 1.25$ mm voxels). Images were resliced so that horizontal sections were oriented parallel to the bicommissural line using ANALYZE. PET images were coregistered to the corresponding MRI image using AIR (Woods et al., 1993). This PET–MRI alignment has a mean error of 2 mm for subcortical structures (Woods et al., 1993; Black et al., 1997). The precision of PET–MRI alignment was verified in three dimensions by visually comparing seven internal points/lines evident on both the PET and MRI images as described in Drevets et al. (2001a,b).

2.3. ROI-based image analysis

To sample radioactive counts in the almond shaped amygdala, an elliptical ROI 6 mm (anterior posterior) by 8 mm (right-to-left) was placed in a single horizontal PET–MRI slice through the center of the basolateral nuclear complex (BLA; comprised of basal, accessory basal and lateral nuclei) using ANALYZE (Mayo Bioengineering, Rochester, MN; Fig. 1). This plane was selected by determining the dorsal–ventral height of the amygdala in sagittal sections, and selecting the horizontal image plane positioned between 35% and 40% of this distance from the ventralmost image plane in which amygdala tissue was evident. In the right–left dimension, the ROI was centered in the grey matter of the amygdaloid complex, so that the lateral border of the ROI was situated about 5 mm from the lateral surface of the amygdala, and the medial border of the ROI was about 5 mm from to the medial surface of the periamygdaloid cortex (Fig. 1). The posterior border of the ROI was formed either by the anterior edge of the temporal horn of the lateral ventricle or the alveus (white matter enveloping

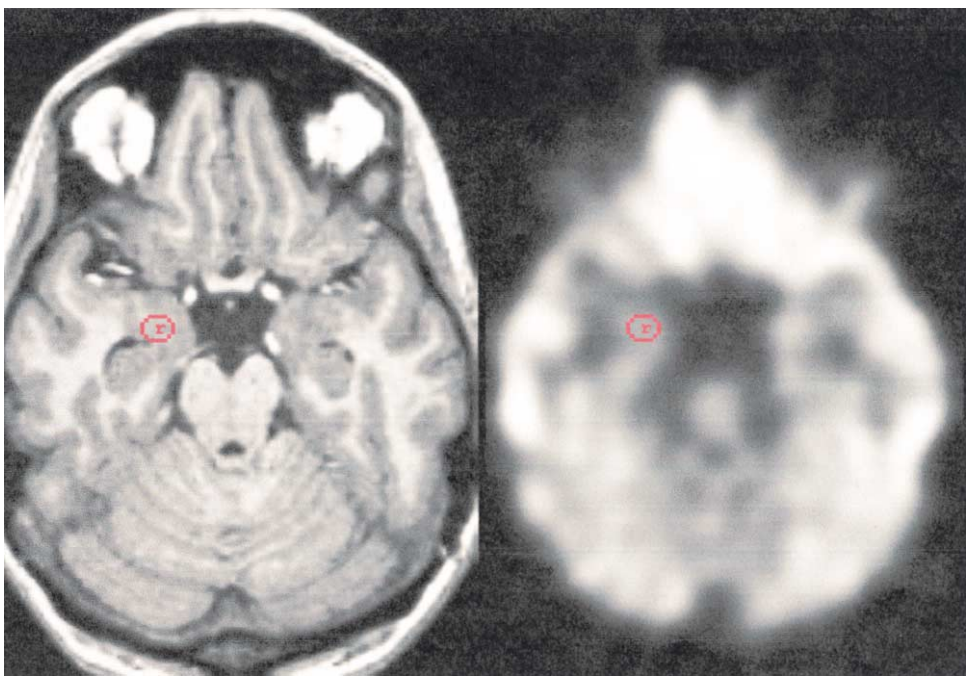

Fig. 1. Aligned anatomical magnetic resonance image (left) and PET image of glucose metabolism (right) showing placement of the ROI in the amygdala. The ROI in which amygdala metabolism was measured was approximately positioned over the basal/accessory basal nuclei, which form the largest nuclear complex within the amygdala. The location of this nuclear complex can easily be approximated in high-resolution MRI images, and ROI centered in the basal nuclear complex are predominantly surrounded by tissue belonging to other portions of the amygdaloid complex (see Discussion). In addition to this technical advantage, previous voxel-by-voxel analysis of our original PET data in FPDD localized the stereotaxic center-of-mass of the area of abnormal CBF to this general area within the amygdala (Drevets et al., 1992; Price et al., 1996; Talairach et al., 1967).

the head of the hippocampus and lining the ependyma of the temporal horn of the lateral ventricle), landmarks which separate the amygdala from the hippocampus (Bronen and Cheung, 1991). To ensure that intergroup differences in this ROI were not accounted by a larger metabolic difference in grey matter outside the amygdala (Links et al., 1996), a *control* ROI was defined in the head of the hippocampus in the same image plane as the amygdala ROI.

The a priori hypothesis was tested by comparing left amygdala metabolism across the control and the three unmedicated depressed groups using ANOVA. Metabolism in the right amygdala and the left and right hippocampi were similarly compared post hoc. Where ANOVA indicated significant main effects of group, specific intergroup contrasts were performed using unpaired *t* tests, with *P* values corrected for the number of comparisons (Bonferroni). Relationships between the amygdala and ipsilateral hippocampal image data were assessed by computing correlation coefficients.

2.4. Plasma cortisol concentrations

Because the plasma cortisol level would be affected both by the stress of the scanning procedure and the circadian pattern of HPA axis activity, scans were scheduled for the same time each morning. Plasma cortisol concentrations were sampled in triplicate in the 10 min prior to FDG infusion, and the average of the three values was used as the cortisol level for each subject. The blood samples were prepared by immediately separating the plasma from whole blood by centrifugation, and the plasma was then stored at -80 °C prior to assay. Plasma cortisol concentrations were measured by radioimmunoassay using a commercial kit from ICN Biomedicals (Costa Mesa, CA) as described elsewhere (Newcomer et al., 1994). The reproducibility of plasma cortisol levels sampled under these conditions was assessed during a repeat scan acquired at least 8 weeks later in a subsample of the controls.

The hypothesis that amygdala metabolism would correlate with these stressed plasma cortisol concentrations was developed after the current replication study had been initiated, so plasma cortisol concentrations were available in only a subset of the subjects described in Table 1. In addition, because the pattern of HPA axis dysfunction observed in atypical depression reportedly differs from that seen in nonatypical depression (Arborelius et al., 1999), unipolar depressives ($n=2$) meeting the atypical depression criteria were excluded from analysis of the relationship between amygdala metabolism and cortisol secretion. This step was implemented a priori to reduce the variability within the data set in order to preserve statistical sensitivity for detecting a relationship between amygdala metabolism and cortisol secretion (it is nevertheless noteworthy that post hoc analysis showed that the correlation between left amygdala metabolism and plasma cortisol levels remained significant when these two subjects were added to the MDD sample). Relationships between cortisol and amygdala metabolism were not examined in the BD-R subjects because some of the psychotropic drugs these subjects were taking may alter glucocorticoid secretion.

The hypothesis that left amygdala metabolism is correlated with the plasma cortisol concentration was tested in the depressed and control samples by computing correlation coefficients. The specificity of this relationship was assessed post hoc by correlating the plasma cortisol levels with metabolism in the right amygdala and the left and right hippocampi. Relationships between left amygdala activity and the clinical ratings were also examined post hoc using nonparametric linear regression analysis.

3. Results

3.1. Subjects

The mean age, gender composition, HDRS score and handedness (Edinburgh handedness inventory; Raczkowski, 1974) of the subject samples appear in Table 1. Except for one subject with a past history of secondary alcohol abuse (she had not abused alcohol for 15 years), none of the MDD subjects had ever met criteria for substance abuse or dependence. Five MDD subjects had never received psychotropic medications (and none was treated with any psychotropic agent in the 3 weeks prior to scanning).

Table 1
Characteristics of the subject samples

Subgroup	Control	MDD	BD	FPDD	DSD	BD-D	BD-R
Sample size	12	21	15	12	9	7	8
Mean age ± S.D.	35 ± 9.8	37 ± 11	35 ± 7.4	36 ± 8.7	40 ± 13	37 ± 9	33 ± 5.6
Percent females	67	76	73	75	78	57	88
Number of left-handed	1	1	1	1	0	1	0
Mean HDRS ± S.D.	0.3 ± 0.5	24 ± 5.5	n.a.	31 ± 6.4	24 ± 4.5	23 ± 6.5	3 ± 3
Mean SAI ± S.D.	9 ± 10	34 ± 13	n.a.	39 ± 13	33 ± 14	28 ± 9.8	17 ± 11
Mean ATQ ± S.D.	3 ± 3	68 ± 26	n.a.	73 ± 21	60 ± 30	51 ± 25	18 ± 14

Abbreviations: ATQ — Automatic Thoughts Questionnaire; BD-D — bipolar disorder, depressed phase; BD-R — bipolar disorder, remitted phase; BD — combines BD-D plus BD-R groups; DSD — depression spectrum disease; FPDD — familial pure depressive disease; MDD — combines FPDD plus DSD groups; HDRS — Hamilton Depression Rating Scale; SSAI — State Anxiety Inventory; n.a. — averaging of clinical ratings across the depressed and remitted BD groups was irrelevant.

Table 2
Normalized (regional/global) metabolism in the primary (in **boldface**) and secondary ROI in the control and currently depressed groups

Region	Control, $n=12$	FPDD, $n=12$	DSD, $n=9$	BD-D, $n=7$
L amygdala	**0.885 ± 0.044**	**0.948 ± 0.058**[a]	**0.909 ± 0.037**	**0.972 ± 0.049**[b,c]
R amygdala	0.926 ± 0.051	0.933 ± 0.051	0.906 ± 0.063	0.982 ± 0.083
L hippocampus	0.969 ± 0.069	0.960 ± 0.066	0.931 ± 0.047	0.973 ± 0.085
R hippocampus	0.955 ± 0.038	0.953 ± 0.067	0.908 ± 0.057[d]	0.969 ± 0.068

Measures compared to test the a priori hypotheses are indicated by **boldface**. Abbreviations: L — left; R — right. Otherwise, as in Table 1.
[a] $P<.05$ relative to the control group, after correction for the number of comparisons.
[b] $P<.01$ relative to the control group, after correction for the number of comparisons.
[c] $P<.05$ relative to the DSD group, without correction for the number of comparisons.
[d] $P<.05$ relative to the control group, without correction for the number of comparisons.

Although none of the MDD subjects was psychotic at the time of scanning, two MDD subject had previously been exposed to antipsychotic drugs and one to lithium.

Three of the seven BD-D subjects were Type I (DSM-IV; American Psychiatric Association, 1994). None of the BD-D subjects had a lifetime history of substance abuse or dependence. Two had previous exposure to antipsychotic agents and two to lithium. One subject had current psychotic features.

Four of the eight BD-R subjects had a history of Type I BD (DSM IV; American Psychiatric Association, 1994). Four BD-R subjects were receiving mood-stabilizing medications at the time of scanning [lithium ($n=1$), divalproex ($n=2$), carbamazepine ($n=1$)]. Two of these four were also taking a phenothiazine antipsychotic drug and one was additionally receiving imipramine. Of the subjects not receiving mood-stabilizing medications, three were unmedicated more than 1 month prior to scanning and one had been taking doxepin (25 mg od) plus alprazolam (0.125 mg po, three to four times per week). The mean Mania Rating Scale score was 2 ± 3 (range 0–8, below the scores expected for hypomania; Young et al., 1978). Three BD-R subjects had a past history of substance abuse (but not within the year prior to scanning).

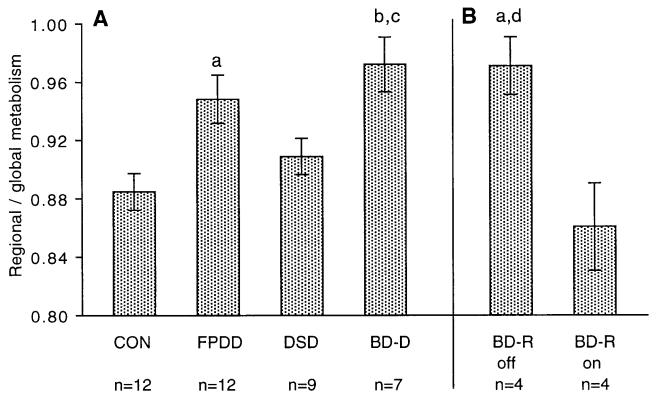

Fig. 2. Mean, normalized glucose metabolism in the left amygdala in: (A) the healthy control and unmedicated, depressed groups studied to test the a priori hypothesis, and (B) the remitted, bipolar, disordered group assessed posthoc. Abbreviations: as in Table 1. "On" and "off" refer to remitted bipolar subjects who were ("on") or were not ("off") receiving mood stabilizer medications. Statistical significance specifiers: (a) P (corrected) $<.05$, relative to the control group; (b) P (corrected) $<.01$, relative to the control group; (c) P (uncorrected) $<.05$, relative to the DSD group; (d) P (uncorrected) $<.05$, relative to the BD-R "on" group.

3.2. Comparisons of regional glucose metabolism between the depressed and control groups

The regional metabolic data appear in Table 2. A significant main effect of group on left amygdala metabolism was demonstrated by ANOVA after covarying for age and gender ($F=6.62$, $df=3,31$, $P<.001$). The left amygdala metabolism was increased 7.1% in the FPDD group [$t=3.0$, $df=22$, P (corrected) $<.05$] and 9.8% in the BD-D group [$t=3.8$, $df=17$, P (corrected) $<.01$] relative to the control group (Fig. 2A). The mean left amygdala metabolism of the DSD group did not significantly differ from those of the FPDD or control groups, but was lower than that of the BD-D group [P (uncorrected) $<.05$].

Post hoc comparisons revealed no significant main effect of group on metabolism in the right amygdala ($F=2.20$, $df=3.36$, $P=.105$), left hippocampus ($F=0.130$, $df=3.30$) or right hippocampus ($F=0.400$, $df=3.30$). Exploratory intergroup t tests revealed a reduction in metabolism in the right hippocampus metabolism (4.9%) in the DSD group relative to the control group [$t=2.1$, $df=19$, P (uncorrected) $<.05$; P (corrected) = n.s.] and a trend toward increased right amygdala metabolism (6.0%) in the BD-D group relative to the control group ($.05<P$ (uncorrected) $<.1$).

The left amygdala metabolism and left hippocampal metabolism were not significantly correlated in either the depressed or the control samples (Table 3). In contrast, the right amygdala metabolism and right hippocampal metabolism were positively correlated in the depressives but not

Table 3
Correlations between regional glucose metabolic measures (r values)

Relations	Controls	All Dep	FPDD	DSD	BD-D
L amyg vs. R amyg	.352	.209	.318	−.396	−.062
L hipp vs. R hipp	.385	.445[#]	.428	.735[#]	.909*
L amyg vs. L hipp	.321	.195	.185	−.049	.066
R amyg vs. R hipp	.159	.681**	.417	.856*	.751[#]

Abbreviations: All Dep — all subjects imaged during a major depressive episode; amyg — amygdala; hipp — hippocampus; L — left; R — right.
* $P<.01$.
** $P<.001$.
[#] $P=.05$.

the controls (Table 3). Metabolisms in the left and right amygdalae were not correlated, but left hippocampal metabolism correlated with right hippocampal metabolism in the depressed sample (Table 3).

3.3. Metabolic measures in the remitted bipolar group

In the BD-R group, the amygdala metabolism (left = 0.916 ± 0.075 and right = 0.958 ± 0.049) was intermediate between the control and the BD-D groups, and not significantly different from either. However, post hoc assessments showed that the left amygdala metabolism was increased in the subjects ($n = 4$) who were not taking mood stabilizer medications relative to the subjects ($n = 4$) who were taking one of these agents (mean metabolism = 0.971 ± 0.040 and 0.861 ± 0.060, respectively; $t = 3.07$; $P < .05$) and to the control subjects ($t = 3.66$, $df = 14$, $P < .005$; Fig. 2B). The mean right amygdala metabolism in the BD-R subjects not receiving mood stabilizers (0.989 ± 0.023) was also significantly higher than that of the controls ($t = 3.38$, $df = 14$, $P < .01$) and trended toward being higher than that of the subjects receiving such medications (0.928 ± 0.052; $t = 2.16$, $.05 < P < .10$).

3.4. Plasma cortisol concentrations

The mean cortisol concentrations were in the stressed range for all groups. This stressed plasma cortisol measure appeared *reproducible* in the eight control subjects scanned on two occasions (separated by a mean interval of 13 ± 4.7 weeks), for whom the mean plasma cortisol level was 15.3 ± 5.9 μg/dl during the first scan session and $15.3 + 3.0$ μg/dl during the second.

For subjects in whom cortisol was sampled, the effect of diagnosis on cortisol level was not significant ($F = 1.18$, $df = 31$, n.s.), with mean plasma cortisol concentrations of 16.8 ± 5.9 μg/dl for the *entire* control group ($n = 11$), 24.6 ± 18.2 μg/dl for the MDD group ($n = 15$), 18.6 ± 6.9 μg/dl for the BD-D group ($n = 6$) and 21.2 ± 7.5 for the BD-R group ($n = 7$). While the glucose metabolism scans and cortisol levels were scheduled at the same time (10 a.m.), variations in subject arrival and preparation, and radioligand delivery caused the scanning and cortisol sampling to vary by up to 2 h. However, the mean cortisol sampling times for the control (10:22 a.m. ± 22 min), MDD (10:45 a.m. ± 26 min) and BD-D groups (10:48 a.m. ± 39 min) did not significantly differ ($F = 2.41$).

Table 4
Correlations (r values) between regional glucose metabolism and plasma cortisol concentrations (the test of the a priori hypothesis is in **boldface**)

Group	n	L amygdala	R amygdala	L hippocampus	R hippocampus
Controls	12	− .23	− .40	− .094	− .15
MDD	**15**	**.69****	− .29	− .040	.010
BD-D	6	.68*	− .21	.59	.23

* $.05 < P < .10$.
** $P < .005$.

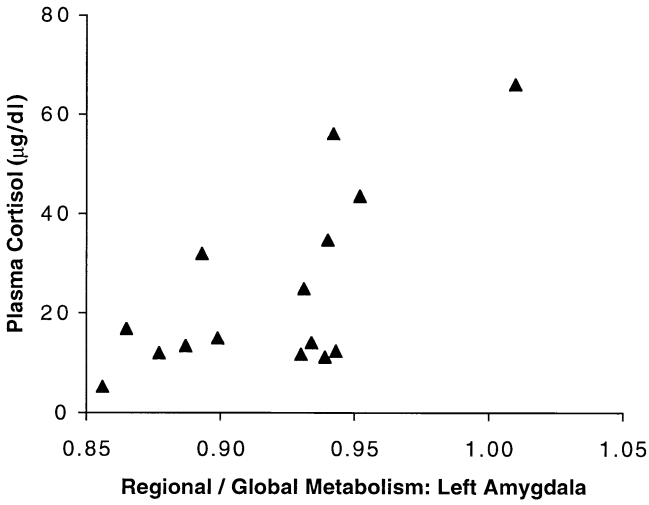

Fig. 3. Relationship between plasma cortisol concentrations measured immediately prior to the PET radiotracer injection and normalized glucose metabolism in the left amygdala for the MDD sample ($n = 15$).

3.5. Correlations between plasma cortisol levels and regional glucose metabolism

The left amygdala metabolism correlated positively with plasma cortisol levels in the MDD and BD-D groups, but not in the control group (Table 4, Fig. 3). Within the MDD sample, the correlation between plasma cortisol levels and left amygdala metabolism was evident in both the FPDD and DSD groups ($r = .72$ and .63, respectively). The correlations computed post hoc between plasma cortisol concentrations and metabolism in the right amygdala and the left and right hippocampi did not approach significance (Table 4).

3.6. Post hoc correlations among amygdala metabolism, age and clinical ratings

Depression severity (HDRS) was positively correlated with the *right* amygdala metabolism in MDD (Table 5). In the entire subject sample, the HDRS scores were positively correlated with left amygdala metabolism ($r = .38$, $P < .05$), although this relationship was not significant within the individual groups (Table 5). Two unanticipated findings were that the left amygdala metabolism was negatively correlated with the SAI and ATQ scores in *controls*. In the mood-disordered samples, neither the SAI nor the ATQ scores correlated with the left or the right amygdala metabolic values (Table 5). The plasma cortisol levels were not correlated with the HDRS or SAI scores in any group.

Age was inversely correlated with left amygdala metabolism in the FPDD group ($r = -.58$, $P < .05$), although this result would not have been significant after corrections for the number of post hoc tests. Age did not significantly

Table 5
Relationship between amygdala metabolism and other clinical variables (r values)

Hemisphere	Left amygdala			Right amygdala		
Clinical rating	HDRS	SAI	ATQ	HDRS	SAI	ATQ
Controls ($n=12$)	n.a.	−.72*	−.94**	n.a.	−.005	−.55
All MDD ($n=21$)	.35	.27	−.00015	.49‡	.15	.13
FPDD ($n=12$)	.31	.10	.11	.52#	.37	.039
DSD ($n=9$)	.39	.36	−.46	.45	−.16	.11
All BD ($n=15$)	.45#	.36	.40	.14	.35	.011
BD-D ($n=7$)	−.15	−.019	.051	−.23	.18	−.39
BD-R ($n=8$)	.66*	.26	.36	.59	.57	.49

Abbreviations: n.a. — not assessed because of too little variability in the clinical rating for the control sample; other abbreviations are as in previous tables.

* $P<.01$, two-tailed test.

** $P<.001$, two-tailed test.

‡ $P=.01$, one-tailed test applied to replicate previous findings of Abercrombie et al. (1998).

# $P<.05$, one-tailed test applied to replicate previous findings of Drevets et al. (1992).

correlate with left amygdala metabolism in the control ($r=-.023$, n.s.), DSD ($r=-.10$) or BD group ($r=-.19$). Age and plasma cortisol levels were not significantly correlated in any group (controls, $r=-.14$; MDD, $r=-.27$; BD, $r=.35$).

In a multiple linear regression model, in which the left amygdala metabolism was the dependent variable, and diagnosis, age and cortisol levels were the independent variables (Model $F=4.9$, $df=4.26$, $P<.005$, $R^2=.43$), diagnosis accounted for most of the variance in metabolism ($\beta=0.61$, $t=4.0$, $P<.0005$). Plasma cortisol, age and gender predicted smaller proportions of the variance ($\beta=0.19$, -0.10 and -0.026, respectively).

4. Discussion

These data replicate the previous finding that neurophysiological activity is abnormally elevated in the left amygdala in FPDD, and extend this finding to glucose metabolism and to bipolar depression. In depressed subjects, the plasma cortisol levels obtained under the stressed conditions of the scanning procedure were positively correlated with left amygdala metabolism, a relationship not evident in healthy controls. In contrast, the corresponding results in the right amygdala and the hippocampus were not significant. Although additional structures were not examined in this study to preserve statistical sensitivity for testing the a priori hypothesis, the amygdala appears to fit within a network of structures involving the orbital and medial PFC, cingulate cortex, thalamus and striatum where metabolic activity is abnormal in the depressed phase of MDD (reviewed in Drevets 2000, 2001).

The accuracy of localizing amygdala measures in the current study was ensured by defining ROI in high-resolution anatomical MRI images, which were then transferred to corresponding PET images that had been coregistered to the MRI image (Fig. 1). This approach also eliminated rater bias since the rater was blind to the PET results during ROI placement. The method used to align PET and MRI images has a mean error of ≤ 2 mm for subcortical structures (Black et al., 1997; Woods et al., 1993). Thus, by situating a small ROI near the center of the amygdaloid complex, we reduced the possibility that misalignment error reduced the specificity of our measures for the amygdala, and decreased the extent to which our measures were influenced by "spilling in" of radioactive counts from structures surrounding the amygdala (Mazziotta et al., 1981; Links et al., 1996).

4.1. Limitations of the methods

4.1.1. Spatial resolution effects

The small size of the amygdala relative to the spatial resolution of PET constitutes a major limitation to the sensitivity for detecting neurophysiological differences in this structure between depressives and controls. Computer simulations that correct measures obtained from a structure with the amygdala's size, geometry and spatial location for the resolution (partial volume) effects of PET suggest that a difference of the magnitude measured between the FPDD/BD-D subjects and controls (Fig. 2) would correspond to an actual increase in CBF and metabolism of 50–70% in the depressives (Drevets et al., 1992; Links et al., 1996). This value is within the expected physiological range, as CBF increases $\sim$50% in the rat amygdala during exposure to fear-conditioned stimuli as measured by tissue autoradiography (LeDoux et al., 1983).

The low spatial resolution relative to the size of the amygdala also reduces the specificity of PET measures of amygdala metabolism because the radioactivity measured from each image voxel (volume element) is influenced by "partial volume averaging" of the radioactivity from nearby voxels (Links et al., 1996; Mazziotta et al., 1981). The 3-D resolution volume for each voxel of 0.5 ml (obtained from the final, reconstructed FWHM resolution of 8 mm) compares to the average volume of the amygdala of 1.22 ± 0.17 ml (left) and 1.11 ± 0.21 ml (right) measured in the controls studied herein using MRI-based morphometry (Ongur et al., 1998). For this reason, metabolism was measured within a small number of voxels from a single image slice (3.375 mm thick) situated deep within the amygdala. This method also eliminated from the average amygdala voxel value those voxels lying on the outer boundary of the amygdala, which would be partly comprised of tissue from structures adjacent to the amygdala (Mazziotta et al., 1981).

Quantitative analysis of PET data is optimal when the size of the structure-of-interest is at least twice as large as the FWHM spatial resolution in all directions (Mazziotta et al., 1981). Unfortunately, the only cerebral grey matter structures for which this principle can be followed using current PET technology are the striatum and thalamus (Talairach et al., 1967). It is nevertheless noteworthy that this principle

is nearly satisfied for our amygdala ROI along the left–right and the dorsal–caudal axes of the amygdala, when considering the amygdala together with the overlying periamygdaloid cortex (which contains cells that largely project to the core amygdala nuclei) (Amaral et al., 1992; Talairach et al., 1967). Thus, in addition to reflecting metabolism within the basal/accessory basal nuclear tissue over which the ROI was centered, the metabolic value was influenced by "spilling in" of radioactivity from the remainder of the basal and accessory basal nuclei anteriorly, dorsally and ventrally, the cortical and central nuclei dorsally, the lateral nucleus laterally, the paralaminar nucleus ventrally, the periamygdaloid cortex anteriorly and medially, and the head of the hippocampus posteriorly (Amaral et al., 1992). Measured activity would also be reduced by "dilutional" effects from the CSF spaces located medially and posteriorly and the white matter situated ventrally and laterally (Links et al., 1996; Mazziotta et al., 1981). The radioactivity measured over our amygdala ROI thus predominantly reflected metabolic activity of the amygdaloid complex grey matter (Links et al., 1996; Drevets et al., 1999).

Because the structure extrinsic to the amygdaloid complex that would have influenced our amygdala measures was the anterior head of the hippocampus, a control ROI was placed in this region to address the specificity of findings to the amygdala. Demonstrating an effect of depression on amygdala metabolism thus depended upon showing that the magnitude of the difference between depressives and controls in the amygdala was not exceeded by an even greater difference in the adjacent hippocampus (Table 2; Drevets et al., 1999, 2001a,b). The ability to assess *relative differences* in radiotracer concentration across conditions in ROI separated by less than the FWHM resolution is central to PET's utility in localizing voxels of maximal difference or correlation in brain mapping studies (Fox et al., 1986; Friston et al., 1991).

Similarly, the specificity of the relationship between *left amygdala* metabolism and cortisol concentrations depended upon showing that this relationship was not accounted for by a greater correlation between left hippocampal metabolism and cortisol (Fig. 3; Drevets et al., 2001a,b). This assessment was of particular relevance because portions of the hippocampus participate in modulating the diurnal pattern of cortisol secretion (Sapolsky et al., 1991; Swanson and Simmons, 1989). However, this role appears to depend upon circadian pacemaker cells, rather than stimulation from afferent projections. Pacemaker cell activity would not be expected to strongly influence the glucose metabolic signal, which is dominated by the energy utilization associated with synaptic transmission within the neutrophil (Magistretti and Pellerin, 1999; Raichle, 1987; Shulman and Rothman, 1998).

Finally, the specificity of findings for the *left* amygdala was supported by the lack of a significant correlation between left amygdala metabolism and left hippocampal metabolism (Table 3). These data suggested that the influence (partial volume effect) exerted by these structures' ^{18}FDG uptake on each other's PET measures was weak. Although *right* amygdala metabolism correlated with right hippocampal metabolism in the depressives, the lack of a similar correlation in the controls raised the possibility that this relationship reflects pathophysiology associated with depression, rather than a partial volume effect.

The correlation between left and right hippocampal metabolism in the depressives was unexpected. These structures are separated by more than twice the FWHM, so this correlation would not reflect a spatial resolution (partial volume) effect. The left and right hippocampi exchange anatomical connections via the hippocampal commissure (crossing fibers which run in the fornix) (Amaral and Insausti, 1990), so this correlation may instead reflect a functional interaction supported by reciprocal connectivity.

4.1.2. Possible influence of abnormalities in mesiotemporal lobe structure in depression

The volume (Sheline et al., 1998; Ongur et al., 1998) and the glia-to-neuron ratio of the amygdala (Bowley et al., 2000) are reportedly *decreased* in MDD relative to healthy samples. In PET images, a reduction in the proportion of grey matter in an ROI relative to the proportion of white matter (which has one-fourth the metabolic activity of grey matter) or CSF (metabolically inactive) can reduce the apparent metabolism by partial volume-averaging effects (Links et al., 1996; Mazziotta et al., 1981). Thus, an abnormal reduction of the amygdala grey matter in MDD would decrease the sensitivity for detecting an elevation of amygdala metabolism relative to controls.

In BD, in contrast, the literature is in disagreement. The amygdala volume has been found to be decreased (Pearlson et al., 1997), not different (Ongur et al., 1998), or increased (Altshuler et al., 1998; Strakowski et al., 1999) relative to healthy controls. The extent to which the conflicting data in BD are explained by medication effects remains unclear, since some mood stabilizer treatments appear to exert neurotrophic/neuroprotective effects (Manji et al., 2001).

Similarly, a reduction in hippocampal volume in depression could reduce measured metabolism in this structure in depressives relative to controls, potentially accounting for the trend toward a reduction in metabolism in the DSD group (Table 2). Subtle reductions in hippocampal volume have been reported bilaterally (Bremner et al., 2000; Mervalla et al., 2000; Sheline et al., 1996; Steffens et al., 2000), only on the right (Swayze et al., 1992), or only on the left (Hirayasu et al., 1998) in MDD and/or BD samples relative to healthy controls. However, most MRI studies found no significant differences in hippocampal volume between mood-disordered and control samples (Ashtari et al., 1999; Axelson et al., 1993; Coffey et al., 1993; Hauser et al., 1989; Pantel et al., 1997; Pearlson et al., 1997; Shah et al., 1998; Vakili et al., 2000; von Gunten et al., 2000). In postmortem studies, histopathological changes in the hippocampus in mood-disordered samples have largely been

limited to the subiculum in BD or predominantly BD samples (Eastwood and Harrison, 2000; Rosoklija et al., 2000). While the subiculum shares extensive anatomical connections with the amygdala (Ongur and Price, 2000), our hippocampal ROI would have been only weakly influenced by this subsection of the hippocampus.

4.1.3. Limitations related to sample size

The small subject sample sizes precluded assessment of gender effects on metabolic activity. Such effects may prove critical to interpreting the laterality of the amygdala metabolic abnormalities in depression because hemodynamic responses in the amygdala to affectively valenced sensory stimuli may be influenced by gender (Cahill et al., 2001). In healthy humans, tasks involving memory for emotionally valenced items have predominantly activated the right amygdala in males (Cahill et al., 1996; Hamann et al., 1999) but the left amygdala in females (Canli et al., 1999, 2000). It is thus unclear whether the laterality of the amygdala metabolic abnormality observed herein in predominantly female depressed samples will differ in a male sample. Notably, in a BD-D sample that contained more males than females, Ketter et al. (2001) found abnormally elevated metabolism only in the *right* amygdala.

4.1.4. Limitations in the assessment of cortisol secretion

The cortisol assays were limited to blood sampled within the minutes prior to injection so that they would correspond to the glucose metabolism measure. The period in which ^{18}FDG is taken up by cells, phosphorylated and trapped intracellularly is largely completed within the first 10 min following ^{18}FDG injection (Huang et al., 1981). The subsequent emission scanning period simply quantitates the radioactivity emitted as ^{18}F decays from the cells in which it was initially trapped. Cortisol levels immediately preceding ^{18}FDG injection were thus expected to be most sensitive to relationships between circulating glucocorticoids and metabolism. However, the assessment of plasma cortisol levels only prior to FDG injection did not address the diurnal pattern of cortisol secretion, and may have increased the variability of the cortisol levels across subjects.

The cortisol concentrations obtained during scanning appeared elevated to the stressed range in all groups, presumably due to the physical and psychological stress of arterial cannulation, venipuncture, immobilization within the thermoplastic mask and other potentially anxiogenic aspects of the scanning process. During exposure to social and/or cognitive stressors, plasma cortisol concentrations increase in anticipation of the stressor onset and remain elevated for over an hour beyond the cessation of stress exposure (Lupien et al., 1997; Young et al., 2000). Since the cortisol values were influenced both by the stress of the scanning procedure and the timing within the circadian pattern of cortisol secretion, we attempted to obtain the cortisol and amygdala metabolic measures at the same time-of-day across subjects. Cortisol levels were sampled after the circadian peak of cortisol secretion when resting cortisol levels are gradually falling (Halbreich, 1987). This stressed cortisol concentration measured to correspond with the ^{18}FDG uptake period proved reproducible in control subjects whose scans were repeated on different days.

In a previous PET study of serotonin type 1A receptor binding, we demonstrated that the plasma cortisol concentrations obtained during PET scanning were significantly higher in depressives than controls (Drevets et al., 1999). In that study, the 29% elevation in cortisol levels in a combined unipolar and bipolar depressive sample relative to a control sample ($P < .05$; Drevets et al., 1999) was similar to the 36% difference between the combined MDD and BD-D (mean = 22.9 ± 15.8 μg/dl) groups vs. the control group (16.8 ± 5.9 μg/dl) in the current study. Nevertheless, neither study assessed the *change* in cortisol secretion in response to PET scanning.

Young et al. (2000) specifically assessed the *change* in plasma cortisol levels in response to the Trier Social Stress test in MDD. The mean plasma cortisol concentrations were higher in the depressives than the controls prior to stress onset, and yet rose by a similar increment in both groups during the stressor. The amount of cortisol secreted in the depressives in response to stress was thus inappropriately high relative to their elevated basal cortisol concentrations (Young et al., 2000). In contrast, studies inducing milder degrees of stress by cognitive challenge alone (i.e., in the absence of explicit social or physical stress) found elevated prestress cortisol levels, but blunted task-induced cortisol elevations, in depressives vs. controls (Trestman et al., 1991; Gotthardt et al., 1995).

4.1.5. Limitations in interpreting the behavioral state in relation to amygdala activity

One possible interpretation of the elevation in amygdala metabolism in depression is that it demonstrates a heightened response to the stress of the scanning procedure, rather than a difference in the true ''resting'' baseline. The metabolic data were acquired as subjects rested with eyes closed because PET measures acquired in this state are stable and reproducible (Ball et al., 1988). Nevertheless, the amount of perceived discomfort elicited by the arterial cannulation procedure, physical restraint and confinement in a small space varied across subjects, and may conceivably have differed between depressives and controls.

However, the following observations argue that the elevated amygdala metabolism in FPDD and BD-D does not simply reflect nonspecific differences in stress or anxiety responses relative to healthy controls: First, amygdala CBF and metabolism have not been found to be elevated *in the eyes-closed, at-rest condition* in anxious subjects with obsessive–compulsive disorder, panic disorder, phobias or posttraumatic stress disorder (reviewed in Drevets and Botteron, 1997; Charney and Drevets, 2001). Second, amygdala metabolism did not correlate with anxiety ratings (Table 5). Third, in imaging studies of healthy humans

scanned under anxiety-provoking conditions, elevations of physiological activity in the amygdala have typically been evident only *during exposure to* emotionally salient sensory stimuli, but not during anxiety elicited by cognitive tasks that do not involve such sensory stimuli (reviewed in Charney and Drevets, 2001). Fourth, Nofzinger et al. (1999) reported that while amygdala metabolism was increased in depressives vs. controls during wakefulness, the increase in metabolism occurring in the amygdaloid complex during rapid eye movement (REM) sleep was also greater in depressives than controls. The amygdala metabolism was thus abnormally increased in MDD even as conscious processing of stressors was presumably dormant.

The latter data converge with the results of other neuroimaging studies indicating that physiological activity in the amygdala is elevated in MDD under a variety of behavioral conditions. In an independent subject sample, Drevets et al. (2001a,b) demonstrated that the mean, left amygdala CBF is higher in depressives than controls while resting with eyes closed, while passively viewing visual objects and while viewing human faces displaying either neutral, sad or happy expression. The same depressed sample showed an exaggerated left amygdala CBF increase during exposure to pictures of sad human faces relative to a healthy control sample, although neither group showed any change in subjective emotional ratings in response to the faces [in contrast, the hemodynamic response of the amygdala to fearful faces was *attenuated* in both these depressed adults (Drevets et al., 2001a,b) and in depressed children (Thomas et al., in press) relative to age-matched controls]. Finally, Kalin et al. (2001) showed that exposure to aversive visual scenes produced a greater hemodynamic response in depressives than controls, and that this difference disappeared following antidepressant drug treatment.

4.2. Possible neurobiological mechanisms underlying amygdala hypermetabolism

Local glucose metabolism is dominated by energy utilization associated with synaptic glutamatergic transmission within the neuropil (Magistretti and Pellerin, 1999; Rothman et al., 1999; Sibson et al., 1998; Shen et al., 1999; Shulman and Rothman, 1998). The sources of afferent glutamatergic transmission that may influence amygdala metabolism originate from local connections between and within amygdala nuclei, and from various distal structures that project to the amygdala (Amaral et al., 1992; Carmichael and Price, 1995; LeDoux, 1987; Ongur and Price, 2000). Amygdala hypermetabolism in depression may thus result from impaired modulation of neural transmission through these projections or increased firing activity in neuronal fields which project to the amygdala (Amaral et al., 1992; Drevets, 2000; Post, 1992; Price et al., 1996).

A potential source of increased synaptic input to the amygdala is the set of PFC areas that share substantial, reciprocal connections with the basal nuclear complex of the amygdala, and have elevated metabolic activity in MDD (Amaral et al., 1992; Carmichael and Price, 1995; Drevets, 2000; Ongur and Price, 2000; Price et al., 1996). While individual amygdala nuclei are too small to resolve using PET, the ROI in which amygdala metabolism was measured was positioned over the basal/accessory basal nuclei for technical and scientific reasons, and was thus most influenced by metabolic activity within these nuclei (Fig. 1). A voxel-by-voxel analysis of our original PET data in FPDD had localized the stereotaxic center-of-mass of the area of abnormal CBF to the vicinity of the basal nuclear complex (Drevets et al., 1992; Price et al., 1996; Talairach et al., 1967). The elevation of amygdala metabolism in FPDD and BD-D could thus reflect increased glutamatergic transmission from the caudal orbital cortex, the anterior insula and the ventral anterior cingulate cortex, which are also hypermetabolic in primary MDD (reviewed in Drevets et al., 1992; Drevets, 2000).

Pertinent to our correlational analyses, the basal nuclear complex is also a site where electrical stimulation increases plasma cortisol concentrations in humans (Fig. 3; Rubin et al., 1966). This latter effect presumably involves anatomical connections between the basal nuclei and the central nucleus of the amygdala (CEA), which sends efferent projections to structures involved in producing the neuroendocrine components of amygdala-mediated emotional responses (Herman and Cullinan, 1997). While the CEA would also be of interest in the current study, it is excessively small and is situated near the dorsal edge of the amygdaloid complex where activity cannot be resolved from activity in neighboring structures using PET.

4.3. Comparison with previous studies

Few published imaging studies of depression have employed experimental methods with adequate sensitivity for detecting physiological abnormalities in the amygdala. Many studies measured CBF using ^{133}Xe inhalation and single photon emission computerized tomography (SPECT) or nontomographic multidetector systems, techniques which provide measures limited to the cortical grey matter lying near the scalp and thus do not sample mesiotemporal lobe structures (Raichle, 1987). Other studies employed SPECT to assess perfusion using radiotracers such as [^{123}I]iodoamphetamine and [^{99}Tc]HMPAO, which have lower spatial resolution than PET, and are less sensitive for detecting areas where CBF increases above resting levels (Drevets et al., 1999). Consequently, few SPECT studies have detected the elevation of amygdala flow in depression (Hornig et al., 1997). Early PET studies used tomographs with limited axial fields-of-view (FOV) that did not sample the entire brain. When such images were analyzed using statistical parametric mapping (SPM) techniques (Friston et al., 1991) that excluded voxels not sampled by all subjects from analysis, the effective FOV was further restricted (to 6 or 7 cm). Many of these studies positioned

the scanner gantry over dorsal brain regions and consequently did not sample ventral areas such as the amygdala (e.g., Bench et al., 1992).

Even in PET studies in which the amygdala was encompassed within the FOV, the common application of voxel-by-voxel image analysis approaches for comparing images from depressives and controls (e.g., Drevets et al., 1992; Friston et al., 1991) has reduced the sensitivity for detecting abnormalities in the amygdala. These techniques require spatial transformation of the primary tomographs into a standardized stereotaxic space so that image data can be averaged across subjects. The spatial transformation algorithms currently employed do not precisely align small structures such as the amygdala across subjects, however. To reduce the effects of this misalignment error, images are blurred (''filtered'') prior to analysis (Poline et al., 1997). Both the reduction of spatial resolution from blurring and the imprecision in overlaying brain structures across subjects decrease sensitivity for detecting abnormalities in small structures such as the amygdala. Thus, Abercrombie et al. (1996) demonstrated that while a positive correlation between depression severity and right amygdala metabolism was evident in MDD when images were analyzed using MRI-based ROI analysis, this relationship was not detected when the same image set was analyzed using SPM.

The reduction in sensitivity for detecting abnormalities of amygdala activity encountered during application of techniques that rely upon spatial transformation may account for our own previous negative study in bipolar depression. Using the stereotaxic method for positioning amygdala ROI that Drevets et al. (1992) employed to detect elevated amygdala CBF in FPDD, Drevets (1995) found no significant difference in the mean, left amygdala CBF between BD-D subjects and healthy controls. Nevertheless, the BD-D sample in that study (Drevets, 1995) contained a higher proportion of Type I and psychotic subjects than the current study, and the extent to which such differences may influence amygdala activity remains unclear.

Inaccuracies involved in spatial transformation procedures may also lead to errors in localizing differences between depressives and controls in voxel-by-voxel analyses. These spatial transformation algorithms generally align external brain surfaces across subjects, but do not specifically align internal structures, assuming instead that the proportionate distances of such structures along orthogonal brain axes is identical across individuals. Regional differences in radioactivity between depressives and controls may thus have errors in their stereotaxic location relative to the actual anatomy, particularly if abnormalities of brain structure exist in depression.

Nevertheless, some studies have detected the abnormal elevation of amygdala activity in depression using voxel-by-voxel analysis techniques. Using such an approach, we previously demonstrated that the area of abnormal CBF in the vicinity of the amygdala had a stereotaxic center-of-mass located within the amygdala (Drevets et al., 1992; Price et al., 1996). Similarly, in PET studies that used SPM to compare unmedicated depressives and healthy controls, abnormal elevations of glucose metabolism have been found in the amygdala in BD-D (Ketter et al., 2001), first-episode MDD (Nofzinger et al., 1999) and elderly MDD (Mentis et al., 1995).

When implementing more anatomically precise MRI-based ROI analysis approaches such as that used herein (Woods et al., 1993), the sensitivity for detecting amygdala abnormalities is critically dependent upon adherence to some technical principles for ROI definition in PET images (Mazziotta et al., 1981). Because of PET's low spatial resolution, it is critical that voxels lying on the outer boundary of a structure be excluded from the average regional voxel value since they will be composed of heterogenous mixtures of neighboring structures, and will increase the influence of radioactivity spilling in from tissues surrounding the structure (Mazziotta et al., 1981; Links et al., 1996). Failure to adhere to this principle may have accounted for the negative study of Abercrombie et al. (1998), in which amygdala ROI were instead defined by outlining the entire amygdala on MRI scans. It is nevertheless noteworthy that Abercrombie et al. (1998) did find a significant, positive correlation between right amygdala metabolism and HDRS scores, consistent with our results in Table 5.

Relationships between regional glucose metabolism and cortisol concentrations have not previously been reported in depression. The positive correlation between glucose metabolism and the stressed plasma cortisol concentrations in depression (Table 4, Fig. 3) therefore requires replication.

4.3.1. Subject selection issues influencing the sensitivity for detecting amygdala hypermetabolism

Another methodological issue that has likely contributed to Type II error in imaging studies of depression has been the tendency of most studies to include subjects who were being medicated with psychotropic drugs. Chronic antidepressant drug administration has been shown to suppress amygdala function and metabolism in humans and experimental animals (see below). Moreover, image data reported for mood-disordered subjects taking antidepressant drugs have generally failed to detect the areas of hypermetabolism identified in unmedicated depressives, and have in some cases shown areas of reduced CBF or metabolism not evident in unmedicated samples (reviewed in Drevets and Botteron, 1997; Drevets, 2000). The preliminary data reported herein in the BD-R sample suggest that mood-stabilizing agents may also suppress amygdala activity (Fig. 2B).

Finally, the extent to which amygdala hypermetabolism may be limited to certain mood-disordered subtypes remains unclear. In the current study, this abnormality was evident in FPDD and BD-D, but statistical power was insufficient to establish whether amygdala metabolism differed between DSD subjects and either control or FPDD subjects. The

FPDD and BD-D criteria are thought to constitute a means for enriching subject samples for the likelihood of having biological abnormalities (Drevets and Todd, 1997; Winokur, 1982). For example, the proportion of depressed subjects who show insulin subsensitivity (a putative sequela of hypercortisolemia) or abnormal suppression of cortisol by dexamethasone was higher in FPDD and BD than in DSD or sporadic depressive disease (SDD; MDD in a subject lacking relatives with depression, mania or alcoholism) (Arana et al., 1985; Lewis et al., 1984; Winokur, 1982; Winokur et al., 1988). Depressives with FPDD also had a higher prevalence of abnormalities of platelet [^{3}H]imipramine binding and sleep EEG than DSD or SDD subjects (Kupfer et al., 1992; Lewis and McChesney, 1985). Finally, depressives with familial mood disorders have been more likely to have elevated CBF and metabolism in the orbital cortex and medial thalamus, and decreased metabolism and cortex volume in the subgenual PFC than depressives without a family history of mood disorders or depressives whose mood disorder arose secondary to other conditions (Drevets, 2000; Drevets et al., 1995; Hirayasu et al., 1998; Kegeles et al., 1999; Ongur et al., 1998). The sensitivity for detecting amygdala hypermetabolism or neuroimaging correlates of hypercortisolemia may thus be increased in FPDD and BD-D samples. Nevertheless, entrance criteria such as responsiveness to sleep deprivation have also successfully identified depressed samples with elevated amygdala metabolism (Wu et al., 1992).

4.4. Relationship between left amygdala metabolism and plasma cortisol secretion

Although correlational evidence cannot identify the mechanism underlying the association between cortisol and amygdala metabolism, direct links between amygdala function and stress-related glucocorticoid release have previously been established. In experimental animals, the adrenocortical responses to stressful stimuli are attenuated by bilateral lesions of the amygdala (Beaulieu et al., 1987; Feldman and Conforti, 1981; Feldman et al., 1994; Gray et al., 1989), and electrical stimulation of the amygdala increases plasma corticosteroid concentrations in humans (Rubin et al., 1966) and experimental animals (Feldman et al., 1995; Weidenfeld et al., 1997). Amygdala activation can putatively increase CRH release by stimulation of CRH-expressing neurons within the amygdala, and by disinhibition of CRH release from the PVN via bisynaptic (double GABAergic) projections from the CEA to the PVN through the bed nucleus of the stria terminalis and through hypothalamic areas adjacent to the PVN (Feldman and Conforti, 1981; Feldman et al., 1994; Gray et al., 1989; Herman and Cullinan, 1997; McEwen, 1995; Raisman and Field, 1971).

The positive correlation between amygdala metabolism and cortisol secretion in the depressed but not the control samples may reflect a failure of negative feedback inhibition in the depressives. In healthy subjects, negative feedback mechanisms would be activated as stressed plasma cortisol levels increase (Young et al., 2000), so that a direct relationship between amygdala metabolism and cortisol concentrations may not be evident. In contrast, major depression is associated with a disturbance of negative feedback inhibition on HPA axis activity (Carroll et al., 1981; Holsboer, 1995; Young et al., 1991, 1993, 2000), which may increase the extent to which cortisol concentrations can be influenced by amygdala activity (Table 4, Fig. 3).

Excessive amygdala activity could conceivably constitute a pathological drive on CRH and cortisol release in MDD and BD (Holsboer, 1995). A central drive on HPA axis activity is evidenced in vivo in MDD subjects by increased CSF levels of CRH, pituitary enlargement and blunted ACTH response to CRH, and postmortem in suicide victims by down-regulation of frontal cortex CRH receptor density and mRNA (Arborelius et al., 1999; Banki et al., 1987; Krishnan et al., 1991; Musselman and Nemeroff, 1993; Nemeroff et al., 1984).

Conversely, amygdala function can also be influenced by glucocorticoids. The glucocorticoid receptor density is high in the human amygdala (Sarrieau et al., 1986), and stimulation of these receptors appears to facilitate the formation of long-lasting associations between sensory stimuli and emotional salience in the amygdala (Roozendaal, 2000). In rats, Corodimas et al. (1994) showed that corticosterone administration potentiates conditioned fear responses to auditory stimuli, which are known to depend upon amygdala function (LeDoux, 1987). In rabbits, intravenous hydrocortisone administration elicits fast electrical activity in the amygdala (Feldman and Davidson, 1965). Memory consolidation related to the formation of associations between sensory stimuli and their emotional significance depends in part upon β and α_1 adrenoreceptor stimulation in the BLA, and norepinephrine release in the amygdala is facilitated by glucocorticoids (Ferry et al., 1999). Corticosterone administration also increases the expression of CRH mRNA in the CEA (Makino et al., 1994; Swanson and Simmons, 1989). Finally, Stutzman et al. (2000) showed that activation of the lateral amygdala via serotonin infusion is dependent upon glucocorticoid receptor stimulation. It is thus conceivable that the positive correlation between amygdala metabolism and cortisol levels may partly reflect an effect of cortisol on the amygdala.

4.5. Relationship among amygdala activity, depressive symptoms and treatment status

The elevation of amygdala metabolism in the BD-R subjects not receiving mood stabilizers relative to the BD-R subjects receiving such agents is an intriguing and novel observation, which requires confirmation in a larger sample. We previously observed that the left amygdala CBF also trended toward being abnormally elevated in the unmedicated, remitted phase of FPDD (Drevets et al.,

1992). Conversely, amygdala metabolism decreased toward normal in MDD during antidepressant drug treatment that both successfully induced symptom remission and prevented relapse on follow-up (Drevets et al., 1996), compatible with preclinical evidence that chronic AD administration has inhibitory effects on amygdala function (Broekkamp and Lloyd, 1981; Cook et al., 1994; Drevets and Raichle, 1992; Drevets et al., 1997; Duncan et al., 1986; Ordway et al., 1991; Wang and Aghajanian, 1980). The preliminary data shown in Fig. 2 similarly suggest that chronic mood stabilizer treatment reduces amygdala metabolism to the normative range in BD. These data support the hypothesis that a persistent elevation of limbic activity underlies the propensity for illness recurrence in mood disorders, and that the modulatory effect of antidepressant and mood-stabilizing drugs on amygdala function constitutes a mechanism through which these agents reduce and prevent pathological mood episodes (Drevets, 1999; Post, 1992). Consistent with the observation that suppression of amygdala metabolism may be critical to the effectiveness of treatment in preventing symptom recurrence, Bremner et al. (1997) reported that antidepressant-medicated, remitted subjects with MDD who relapse during tryptophan depletion have a higher baseline amygdala metabolism than those who do not relapse.

The positive correlation between neurophysiological activity in the right amygdala and depression severity rated by HDRS scores may reflect the amygdala's role in organizing multiple aspects of emotional/stress responses (Drevets, 2001). The amygdala is involved in the acquisition, consolidation and expression of emotional/arousing memories (Buchel et al., 1998; Cahill et al., 1996, 2001; Canli et al., 2000; LeDoux, 1987; LaBar et al., 1998) and the recognition of emotionally salient social information in facial expression (Adolphs et al., 1994; Thomas et al., 2001; Drevets et al., 2001a,b; Morris et al., 1996) and spoken language (Scott et al., 1997). In humans, bursts of EEG activity have been recorded in the amygdala during recollection of specific emotional events (Halgren, 1981), and electrical stimulation of the amygdala can evoke emotional experiences (fear, anxiety, dysphoria; Gloor et al., 1982) and recall of emotionally charged life events (Brothers, 1995). Amygdala dysfunction in mood disorders could thus conceivably alter the evaluation of and memory consolidation related to the emotional significance of sensory or social stimuli.

4.6. Future directions

Progressive advances in the development of neuroimaging technology will enable in vivo investigation of human amygdala function at increasingly higher spatial resolution. The ability to measure metabolism in the amygdala and the remainder of the limbic–cortical–striatal–pallidal–thalamic circuitry in the absence of major partial volume effects may ultimately permit sensitive and specific classification of individual depressed subjects. Combining such techniques with pharmacological manipulations of glucocorticoid synthesis and receptor function may elucidate the relationship between amygdala function and HPA axis activity. Finally, inclusion of genotype data for proposed disease susceptibility loci and relevant pharmacogenetic mechanisms in future imaging studies may enable clarification of the complex relationships between abnormal amygdala function and depression, mania and illness recurrence, and the role of modulating amygdala activity during treatment for these conditions.

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