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Estimates of Serotonin and Norepinephrine Transporter Inhibition in Depressed Patients Treated with Paroxetine or Venlafaxine

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Paroxetine and venlafaxine are potent serotonin transporter (SERT) antagonists and weaker norepinephrine transporter (NET) antagonists. However, the relative magnitude of effect at each of these sites during treatment is unknown. Using a novel blood assay that estimates CNS transporter occupancy we estimated the relative SERT and NET occupancy of paroxetine and venlafaxine in human subjects to assess the relative magnitude of SERT and NET inhibition. Outpatient subjects ($N = 86$) meeting criteria for major depression were enrolled in a multicenter, 8 week, randomized, double-blind, parallel group, antidepressant treatment study. Subjects were treated by forced-titration of paroxetine CR (12.5–75 mg/day) or venlafaxine XR (75–375 mg/day) over 8 weeks. Blood samples were collected weekly to estimate transporter inhibition. Both medications produced dose-dependent inhibition of the SERT and NET. Maximal SERT inhibition at week 8 for paroxetine and venlafaxine was 90% (SD 7) and 85% (SD 10), respectively. Maximal NET inhibition for paroxetine and venlafaxine at week 8 was 36% (SD 19) and 60% (SD 13), respectively. The adjusted mean change from baseline (mean 28.6) at week 8 LOCF in MADRS total score was −16.7 (SE 8.59) and −17.3 (SE 8.99) for the paroxetine and venlafaxine-treated patients, respectively. The magnitudes of the antidepressant effects were not significantly different from each other (95%CI −3.42, 4.54, $p = 0.784$). The results clearly demonstrate that paroxetine and venlafaxine are potent SERT antagonists and less potent NET antagonists *in vivo*. NET antagonism has been posited to contribute to the antidepressant effects of these compounds. The clinical significance of the magnitude of NET antagonism by both medications remains unclear at present.
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INTRODUCTION

The majority of antidepressants used to treat major depressive disorder (MDD) are primarily antagonists at the human serotonin (SERT) and/or norepinephrine transporters (NET) (Owens *et al*, 1997, 2001; Nemeroff and Owens, 2002). The percentage of patients exhibiting response and remission to either selective norepinephrine uptake inhibitors or SSRIs in an acute, randomized, double-blind clinical trial is approximately 60% and 35–40%, respectively (Lieberman *et al*, 2005; Nemeroff and Schatzberg, 2006).

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Recently, there is an increasing appreciation that antidepressant transporter selectivity is relative and is dependent upon the concentration of drug at the transporter, which is ultimately related to dose (Owens, 2004). There is continuing controversy as to whether a combination of both SERT and NET inhibition represents a more effective antidepressant treatment than drugs that target a single neurotransmitter system, particularly for severe or refractory depression (Seth *et al*, 1992; Nelson, 1998; Thase *et al*, 2001; Nemeroff, 2006). In an open-label study, combination treatment with desipramine, a relatively selective inhibitor of the NET, and the SSRI fluoxetine led to a more rapid onset of antidepressant efficacy than either treatment alone (Nelson *et al*, 1991). More recently, this group reported that the combination of fluoxetine plus desipramine was significantly more likely to result in remission than either drug alone in a double-blinded study (Nelson *et al*, 2004). Similarly, a combination of nortriptyline and fluoxetine or sertraline was more effective than the use of one of these

agents alone in ameliorating treatment-resistant depression (Seth *et al*, 1992). A pooled analysis of all of the Wyeth-sponsored venlafaxine clinical trials revealed that remission rate differences for venlafaxine *vs* SSRI therapy were small but statistically significant, largely attributed to venlafaxine-fluoxetine differences (Nemeroff *et al*, 2008).

Venlafaxine is classified as a dual SERT/NET inhibitor that does not posses the anticholinergic, antihistaminergic, and antiadrenergic properties of the dual reuptake inhibitor tricyclic antidepressants such as imipramine and amitriptyline. Multiple investigators have demonstrated that venlafaxine and its active metabolite, *O*-desmethylvenlafaxine (ODV), are relatively weak inhibitors of the NET *in vitro* (Owens *et al*, 1997; Tatsumi *et al*, 1997; Beique *et al*, 1998). However, partly because of its low serum protein binding, it is widely accepted that venlafaxine does possess dual uptake blocking properties *in vivo*, particularly in the upper range of dosage.

Paroxetine has been classified as an SSRI on the basis of its very high affinity for the SERT ($K_i = 65$ pmol/l), although evidence from several laboratories has indicated that paroxetine also shows moderate affinity for the NET ($K_i = \sim 40$–85 nmol/l) (Owens *et al*, 1997, 2001; Tatsumi *et al*, 1997; Beique *et al*, 1998). Laboratory animal studies have confirmed that after systemic administration, paroxetine partially inhibits the NET in rat brain homogenates (Owens *et al*, 2000). Thus, at higher concentrations/doses paroxetine loses its SERT selectivity and, like venlafaxine, may act as a dual serotonin/norepinephrine uptake inhibitor (SNRI). Indeed, our group has previously reported that paroxetine, beginning at a dose of 40 mg/day, partially inhibits the NET while producing $>80\%$ SERT inhibition (Gilmor *et al*, 2002). That initial study, a subsequent small study (Davidson *et al*, 2005), and the present one used a modified monoamine uptake assay that measures SERT and NET inhibition using serum obtained from blood samples of patients treated with escalating doses of the medication under investigation. This method maintains the important equilibrium between free and serum protein-bound drug, hence modeling *in vivo* conditions where only free drug is generally considered accessible to the brain and clinically relevant sites of action.

The present study tested the hypothesis that both venlafaxine and paroxetine partially inhibit the NET while producing robust ($>80\%$) SERT inhibition in a dose-dependent manner in subjects with MDD.

MATERIALS, PATIENTS, AND METHODS

Subjects

Patients were enrolled at 7 clinical research centers in the United States from April to December, 2003. INC Research Inc. (Raleigh, NC) provided managerial oversight of the clinical research centers. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (1996). The protocol and informed consent were approved by the Institutional Review Boards or Ethics Committees before each center's initiation.

Male and female subjects (18–65 years) meeting diagnostic criteria for MDD were eligible (American Psychiatric Association, 1994). The diagnosis of MDD was made by the principal investigator using the Mini International Neuropsychiatric Interview (MINI)—a structured diagnostic interview for DSM-IV (Sheehan *et al*, 1998). A total score of $\geqslant 20$ on the Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) was required at screening and baseline. Patients were excluded if they had a clinically predominant axis I disorder other than MDD. Other key exclusion criteria were: history of unresponsiveness to either paroxetine or venlafaxine or exhibited prior hypersensitivity/intolerance to either paroxetine CR or venlafaxine XR, substance abuse/dependence, prior non-response to SSRIs, suicidal/homicidal risk, concurrent psychotherapy or psychotropic pharmacotherapy, or any serious medical condition or clinically significant finding in the screening or baseline evaluation that would preclude the administration of paroxetine CR or venlafaxine XR.

Patients were excluded if they required concomitant therapy with psychoactive medication or patients who have taken other psychoactive medication within the time frames specified below prior to the screening visit: antidepressants other than MAOIs or fluoxetine (eg, tricyclic antidepressants, SSRIs, and NSRIs), lithium and oral antipsychotics—14 days ; hypnotics, benzodiazepines, and all other sedatives (including chlorpheniramine and other sedating antihistamines)—14 days; fluoxetine, MAOIs—4 weeks; depot neuroleptics—12 weeks; any CNS-active herbal preparations/supplement (eg, St John's wort, kava kava, etc.)—14 days.

Study Design

This GSK supported collaborative research trial (study 826) was a prospective, multicenter, 8-week, randomized, double-blind, parallel group, forced-titration of paroxetine CR (12.5–75 mg/day) or venlafaxine XR (75–375 mg/day). Patients meeting eligibility criteria at baseline were randomized (1 : 1) to receive over-encapsulated PAR or VEN tablets using a computer-generated randomization list. For the first week, patients received PAR 12.5 mg/day or VEN 75 mg/day. Patient's dose was force titrated upward at weekly and bi-weekly intervals to a maximum dosage of either PAR 75 mg/day or VEN 375 mg/day as shown in Table 1. Patients unable to tolerate dose increases were withdrawn from the study. On-treatment study assessments were scheduled at the end of weeks 1, 2, 3, 4, 5, 6, 7, and 8 or upon early withdrawal. Assessments of efficacy, safety, and tolerability were conducted at each visit. Weekly blood samples were obtained for measuring serum drug concentrations and for transporter occupancy measurements (see below). Drug concentrations were analyzed by an experienced laboratory (Quest Diagnostics Clinical Trials, Los Angeles, CA). The limit of detection for paroxetine, venlafaxine, and ODV was 10 ng/ml.

Efficacy Assessments

The primary objective was to estimate and compare PAR- and VEN-induced NET and SERT inhibition in patients with MDD using an *ex vivo* method described previously (Gilmor *et al*, 2002; Davidson *et al*, 2005); details below). The relationship between percentage inhibition of the NET and the SERT and medication dosage and serum drug

Table 1 Subject Treatment and Dosage Over Time

Treatment	Daily dosage							
	Dosage level 1	**Dosage level 2**	**Dosage level 3**		**Dosage level 4**		**Dosage level 5**	
							375 mg	375 mg
					300 mg	300 mg	$n=33$	$n=32$
			225 mg	225 mg	$n=37$	$n=36$		
		150 mg	$n=38$	$n=38$				
Venlafaxine XR	75 mg	$n=38$						
	$n=44$						75 mg	75 mg
					62.5 mg	62.5 mg	$n=33$	$n=32$
			50 mg	50 mg	$n=34$	$n=34$		
		25 mg	$n=37$	$n=34$				
Paroxetine CR	12.5 mg	$n=39$						
	$n=42$							
Screen/baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8

Each subject received five different dosage levels of venlafaxine XR or paroxetine CR over an 8-week treatment period. The weeks of treatment are shown at the bottom of each column. The number of subjects (*n*) remaining within the study at each time point are listed for each medication at each week.

concentration were determined. Clinical efficacy measures were secondary objectives in this study. The key measure of efficacy was change from baseline in MADRS total score at week 8 last observation carried forward (LOCF) endpoint. Additional measures of efficacy (also based on week 8 LOCF endpoint) were: proportion of Clinical Global Impression global improvement item (CGI-I) responders (Guy, 1976) defined as a score of 1 (very much improved) or 2 (much improved change from baseline) on the CGI-S (Guy, 1976). *Post hoc* analyses were conducted to determine the proportion of responders (defined as a $\geqslant$50% reduction in MADRS total score) and remitters (defined as a MADRS score $\leqslant$10) in each treatment group. A psychiatrist, clinical psychologist, or psychometrician with at least 2 years experience in treating patients conducted all efficacy assessments.

Antidepressant Response

All patients who were randomized, received at least one dose of study medication and had at least one post-baseline efficacy assessment, were included in the modified intent-to-treat (ITT) efficacy analyses. Exploratory analysis of clinical efficacy data (secondary objectives in this study) was performed using *t*-tests (two-sided, $P<0.05$ level of significance). Analysis of change from baseline in MADRS was performed separately at each post-baseline time point using the *proc glm* procedure in SAS® version 8.2. There was no adjustment for any covariates or any repeated measures analysis. Patients withdrawing before week 2 without MADRS assessments are not included in the analyses of the MADRS. The analyses on observed data were repeated using the LOCF approach to impute missing values. Subjects with a CGI global improvement rating of 'much improved' or 'very much improved' were categorized as responders. The responder rates at each post-baseline visit (study weeks 1 through 8) were compared between the treatment groups in an exploratory fashion. The comparisons were performed using the Fisher's Exact Test (two-sided). In addition, 95% confidence intervals (CI) for the differences in responder rates were estimated using the normal approximation to the binomial distribution. Analyses were performed using *proc freq* in SAS® version 8.2. All patients randomized at baseline were used in the ITT safety analyses. The number (percentage) of patients in each treatment group (both combined and by dosage level) with treatment emergent adverse experiences were compared for overall incidence.

Ex vivo [^{3}H]-Serotonin and [^{3}H]-Norepinephrine Uptake and Analysis

The assay method has been described in detail previously (Gilmor *et al*, 2002). Positive control sample wells consisted of normal human serum containing either 300 nmol/l paroxetine or 3 μmol/l desipramine for the [^{3}H]-serotonin and [^{3}H]-norepinephrine uptake assays, respectively.

Individual points from patient data were obtained by calculating the mean of replicate values (5–7; minimum of 3) from a given sample as the percent baseline of the pre-drug treatment sample mean (baseline) for that individual. An individual replicate was excluded from mean sample calculation if it was significantly different than all other replicates (eg >3 SD away from sample mean). Percent occupancy is calculated as 100−percentage of baseline. Concentration-uptake curves were generated with the nonlinear, curve-fitting program PRISM 3.0 (GraphPad, San Diego, CA) by using a one-site competition curve to describe drug–transporter interactions (Kenakin, 1997). Because drug serum concentrations vary widely among patients at identical dosages, competition curves were generated by treating the data points from all analyzed individuals in each treatment group as individual data points (ie, a certain amount of transporter blockade at a given serum drug concentration), rather than generating a curve for each patient and then averaging these curves. Means are presented with standard deviations.

Statistical Analysis

INC Research, Inc. (Raleigh, NC) provided professional statistical analysis of the efficacy and tolerability data. Analysis of all serum data was conducted by the Emory University investigators using SigmaStat 3.0 and SPSS (SPSS Inc., Chicago, IL) and PRISM 3.0 (GraphPad, San Diego, CA).

RESULTS

Serum Concentrations and Estimated Transporter Inhibition

As shown in Figure 1, mean serum concentrations of paroxetine and combined venlafaxine plus ODV increased with increasing dosage ($P<0.001$; one-way repeated measures ANOVA). Because venlafaxine and ODV have essentially identical affinities at the SERT and NET (Owens *et al*, 1997), their serum concentrations were combined. As shown in the insets of Figure 1, there is a high degree of inter-individual variability in the resultant steady-state serum concentrations produced by identical dosages.

As predicted from *in vitro* studies of affinity, both medications dose-dependently inhibited the SERT (Figure 2 top) and NET (Figure 2 bottom) (one-way repeated measures ANOVA; $P<0.001$ for each medication and for both SERT and NET). At the doses tested in this particular study, maximal mean SERT inhibition was estimated to be 90% (SD 7) and 85% (SD 10) in the paroxetine and venlafaxine groups, respectively. At week 8, the maximal inhibition of the SERT produced by paroxetine was significantly greater than that produced by venlafaxine (Figure 2; $p=0.038$, Student *t*-test with Welch's correction, $t=2.15$, $df=42$, 95% CI 9.81–0.30). Similarly, maximal mean NET inhibition was estimated to be 36% (SD 19) and 60% (SD 13) in the highest dosage paroxetine and venlafaxine treatment groups at week 8, respectively (Figure 2; $P<0.001$, Student *t*-test with Welch's correction, $t=5.31$, $df=46$, 95% CI 14.7–32.7).

Because transporter inhibition is directly related to free drug concentrations within the extracellular water surrounding neurons and that this is more closely related to serum drug concentrations than to drug dosage, we calculated the relationship between serum drug concentrations and SERT and NET inhibition using all available data points and classic drug-receptor curve fitting software (one-site competition curve, PRISM 3.0 (GraphPad, San Diego, CA)) (Figure 3). As shown in Figure 3, the vast majority of all data points, which included all patient samples in which a quantifiable serum drug concentration could be attained, revealed considerable (>70%) SERT inhibition. These data reveal that the inhibition constant (K_i) value, which represents the mean serum concentration necessary to inhibit 50% of available transporters, is 9 ng/ml (23 nmol/l; 95% CI 21–25 nmol/l) and approximately 85 ng/ml (315 nmol/l; 95% CI 294–338 nmol/l) for paroxetine and total venlafaxine concentrations, respectively. Although neither medication is as efficacious in inhibiting the NET compared to the SERT, both paroxetine and venlafaxine produced a concentration-dependent inhibition of the NET (Figure 3). For NET inhibition, K_i values were serum concentrations of 227 ng/ml (690 nmol/l; 95% CI 590–805 nmol/l) and approximately 325 ng/ml (1 204 nmol/l; 95% CI 1126–1288 nmol/l) for paroxetine and 'total venlafaxine', respectively.

Treatment Response

In total, 86 patients were randomized, of which 55 (64.0%) were female subjects and 31 (36.0%) were male subjects. Because five of the randomized patients did not have a post-baseline efficacy assessment, the evaluable population (modified ITT) consisted of 81 (40 paroxetine CR patients and 41 venlafaxine XR patients) patients. Demographic and baseline characteristics of the ITT population are available upon request.

The mean MADRS total score at baseline was 28.6 (SD 5.05) for PAR and 28.6 (SD 5.97) for VEN, indicative of moderate-to-severe MDD symptomatology. This level of severity was also reflected in the baseline CGI-S ratings for PAR (mildly Ill = 2.5%, moderately Ill = 62.5%, markedly Ill = 27.5%, and severely or among the most extremely Ill = 7.5%) and VEN (mildly Ill = 0%, moderately Ill = 56.1%, markedly Ill = 31.7%, and severely or among the most extremely Ill = 12.2%).

A total of 74.4% (64/86) of patients (ITT population) completed the 8-week, double-blind phase. The percentage of patients who withdrew for any reason was 27.3% (12/44) in the VEN group and 23.8% (10/42) in the PAR group. The proportion of patients withdrawn due to an AE in the VEN group was 9.1% (4/44), compared with 4.8% (2/42) in the PAR group.

Overall, at week 8, 76% (32/42) of PAR patients received the maximum daily dose (75 mg per day) compared with 73% (32/44) of VEN-treated patients who received the maximum daily dose (375 mg per day). The adjusted mean change from baseline at week 8 LOCF endpoint in MADRS total score was −16.7 points (SE 8.59) for patients randomized to PAR and −17.3 points (SE 8.99) for those randomized to VEN. The adjusted mean difference between the treatment groups, 0.60 points, was not statistically significant (95% CI −3.42, 4.54, $p=0.784$) (Table 2). At week 8, the percent CGI-I response rate (LOCF) was 78.9% for VEN and 67.5% for PAR. This difference was also not statistically significant (95% CI −30.9, 8.0; $p=0.312$).

Two *post hoc* LOCF analyses (using a two-tailed Fisher's Exact Test) were conducted: (1) the proportion of responders in each treatment group achieving at least a 50% reduction in MADRS total score (from baseline), and (2) the proportion of patients in remission at week 8 LOCF defined as a MADRS score less than or equal to 10. Although the VEN group showed slightly greater numerical improvement for both response (VEN *vs* PAR 71.1 *vs* 64.9%, $p=0.626$) and remission (VEN *vs* PAR 63.2 *vs* 45.9%, $p=0.167$), the differences were not statistically significant.

Overall, similar proportions of patients on PAR and VEN had at least one treatment emergent AE (81.0% [34/42] for PAR *vs* 81.8% [36/44] for VEN). Most of the AEs were mild to moderate in intensity and did not lead to withdrawal (details available upon request).

Estimated Transporter Inhibition and Treatment Response

The present study was neither primarily designed nor powered to determine the relationship between the magnitude

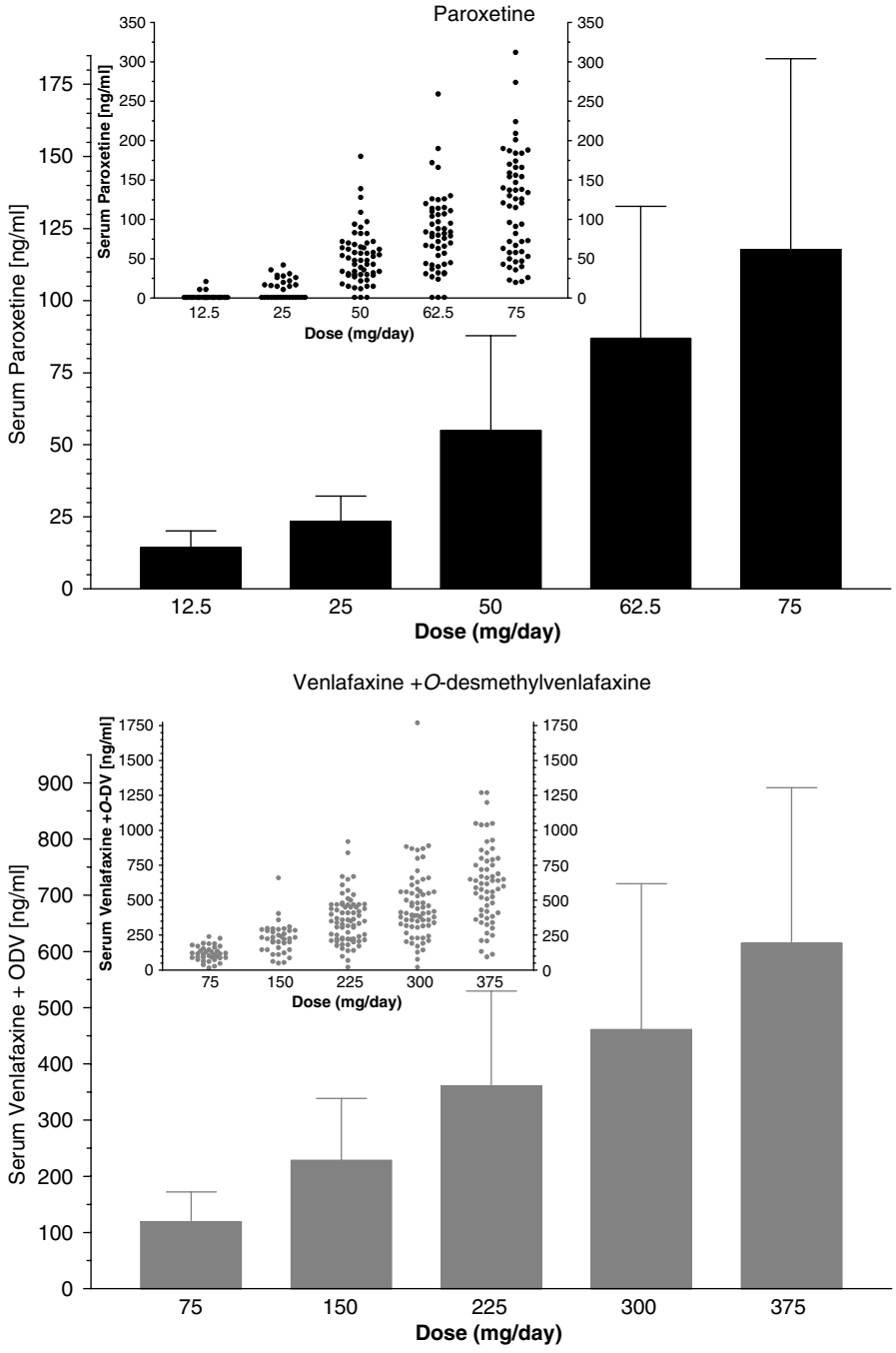

Figure 1 Paroxetine and combined venlafaxine and *O*-desmethylvenlafaxine (ODV) serum concentrations as a function of dose. Following at least 1 week of therapy at each dose, mean serum concentrations (mean ± SD) increased as a function of dose. However, there is wide inter-individual scatter at identical doses (inset). Data points in the scatterplot at 0 ng/ml represent samples below the analytic limit of detection (10 ng/ml; Quest Diagnostics) and may represent low concentrations associated with low doses or non-compliance. There were no differences between the same dose of drug at different time points (eg, weeks 7 and 8; one-way ANOVA with Tukey *post-hoc* test). Data from identical doses at different time points were collapsed into single bars for display purposes. Bar data only used data points with measurable serum concentrations. Log[mol/l] serum concentrations of paroxetine = log([ng/ml]/329 400 000). Venlafaxine and ODV have essentially identical affinities at the SERT and NET (Owens *et al*, 1997) and can be combined on a molar basis to yield a total active amount of drug. Log[mol/l] serum concentrations of combined venlafaxine + ODV $\approx$ log([ng/ml]/270 400 000).

of individual, or combined, SERT or NET inhibition and clinical response or remission. Nevertheless, we plotted the magnitude of SERT or NET inhibition and treatment response in the subjects who both completed the full 8-week treatment and provided a serum sample for SERT and NET occupancy at this time point. No significant interactions (two-way ANOVA) were observed between SERT or NET inhibition and treatment response to paroxetine or venlafaxine (Figure 4). Similarly, no differences in transporter inhibition and treatment response were observed when all subjects were examined on the basis of SERT and NET inhibition only (bottom graph of Figure 4).

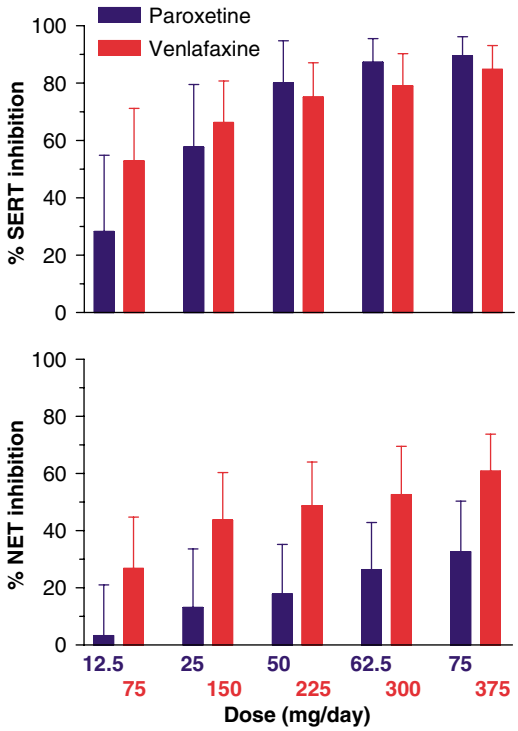

Figure 2 Estimated CNS SERT and NET inhibition by paroxetine and venlafaxine. Percent SERT inhibition (ie, occupancy) was dose-dependently increased by both paroxetine and venlafaxine (mean ± SD; one-way repeated measures ANOVA, $P<0.001$, $F=89.0$ [paroxetine], $F=53.7$ [venlafaxine]). There were no differences between the same dose of drug at different time points (eg, weeks 7 and 8). Within each drug, the lowest dose was significantly different than every other dose and doses separated by 25 mg/day (paroxetine) or 150 mg/day (venlafaxine) were significantly different than each other ($P<0.05$; Tukey test). Data from identical doses at different time points were collapsed into single bars for display purposes only. Although the difference was only 5% occupancy, direct comparison of the week 8 data revealed that paroxetine 75 mg/day produced greater SERT inhibition than venlafaxine 375 mg/day ($p=0.002$, Student *t*-test with Welch's correction, $t=2.15$, $df=46$, CI 9.81–0.30). These data are based upon dosage only and include samples for which paroxetine was below the limit of detection. Percent NET inhibition (ie, occupancy) was dose-dependently increased by both paroxetine and venlafaxine (mean ± SD; one-way repeated measures ANOVA $P<0.001$, $F=15.5$ [paroxetine], $F=33.1$ [venlafaxine]). There were no differences between the same dose of drug at different time points (eg, weeks 7 and 8). Within each drug, the lowest dose was significantly different than every other dose and doses separated by 25 mg/day (paroxetine) or 150 mg/day (venlafaxine) were significantly different than each other ($P<0.05$; Tukey test). Data from identical doses at different time points were collapsed into single bars for display purposes only. Direct comparison of the week 8 dose of each drug used in this study revealed that venlafaxine 375 mg/day produced greater NET inhibition than paroxetine 75 mg/day ($P<0.001$, Student *t*-test with Welch's correction, $t=5.3$, $df=46$, 95% CI 14.7–32.7). These data are based upon dosage only and include samples for which paroxetine was below the limit of detection.

DISCUSSION

Transporter and receptor occupancy has become an increasingly important area of research in understanding the therapeutic mechanisms of antidepressant and antipsychotic drugs. Over the past several years, Meyer and colleagues have performed PET scans of SERT availability in patients treated with SSRIs (Meyer *et al*, 2001, 2004). Of particular interest, dose- and concentration-occupancy curves revealed that doses commonly considered therapeutically effective produced approximately 80% SERT occupancy. This was consistent across all SSRIs. Moreover, some subjects clearly had lower SERT occupancy, though the therapeutic outcome of those individuals was not reported. Meyer and colleagues found no relationship between striatal occupancy and percent change in the HAMD score from scan 1 (pre-drug baseline) to scan 2 (steady-state drug treatment); however, it should be noted that striatal occupancy following this standardized drug treatment occurred across a small window rather than a large (eg, 0–100% occupancy) window in which clear relationships between occupancy, or thresholds of necessary occupancy, and variations in clinical response may be more easily observed. The present studies are the first to measure both SERT and NET occupancy provisionally needed for efficacy in a larger population of depressed subjects. These data may be extremely valuable in monitoring patient compliance, the need for dosage adjustment and, in the case of adequate occupancy without therapeutic response, information that provides a rational decision to switch medication class or initiate other treatment options. Unfortunately, the costs and limited availability of PET facilities renders SERT occupancy measurements impractical for the vast majority of physicians.

We have developed a unique method in which to measure the magnitude of 5-HT or norepinephrine transporter occupancy in antidepressant-treated patients by exposing cells transfected with the human SERT or NET to the patient's serum after steady-state is attained (Gilmor *et al*, 2002; Davidson *et al*, 2005). This allows for a reliable and quantitative method to estimate in any given patient the magnitude of SERT or NET inhibition. Because only the unbound, 'free' fraction of the drug interacts with the transfected cells, differential protein binding of antidepressants is not a confound in these studies.

It is assumed that the free drug concentration as assessed with this novel method is approximately the same drug concentration available to enter the brain, and therefore corresponds to brain extracellular drug water concentrations (Frazer, 2001). Classical receptor pharmacological theory predicts that receptor (or transporter) occupancy and biological action is a concentration-dependent phenomenon (Kenakin, 1997). Depending upon the affinity of each individual drug, there is a direct correlation between receptor occupancy (a function of drug concentration) and biological response. The shapes of these curves may differ depending upon the specific properties of the individual drug (eg, full *vs* partial agonist) and whether there exist 'spare' receptors. Until recently, little or no data were available regarding the magnitude of SERT blockade necessary for an antidepressant response in humans. Moreover, the percentage of transporter occupancy that occurs as a function of antidepressant dose or serum drug concentration was not known.

Knowledge of the concentration of antidepressants in the extracellular fluid surrounding neurons could theoretically be used to estimate transporter blockade based upon the results of *in vitro* binding studies (Owens *et al*, 1997, 2001; Tatsumi *et al*, 1997; Beique *et al*, 1998) and central nervous system (CNS) pharmacokinetics (Gjedde *et al*, 2000). It is

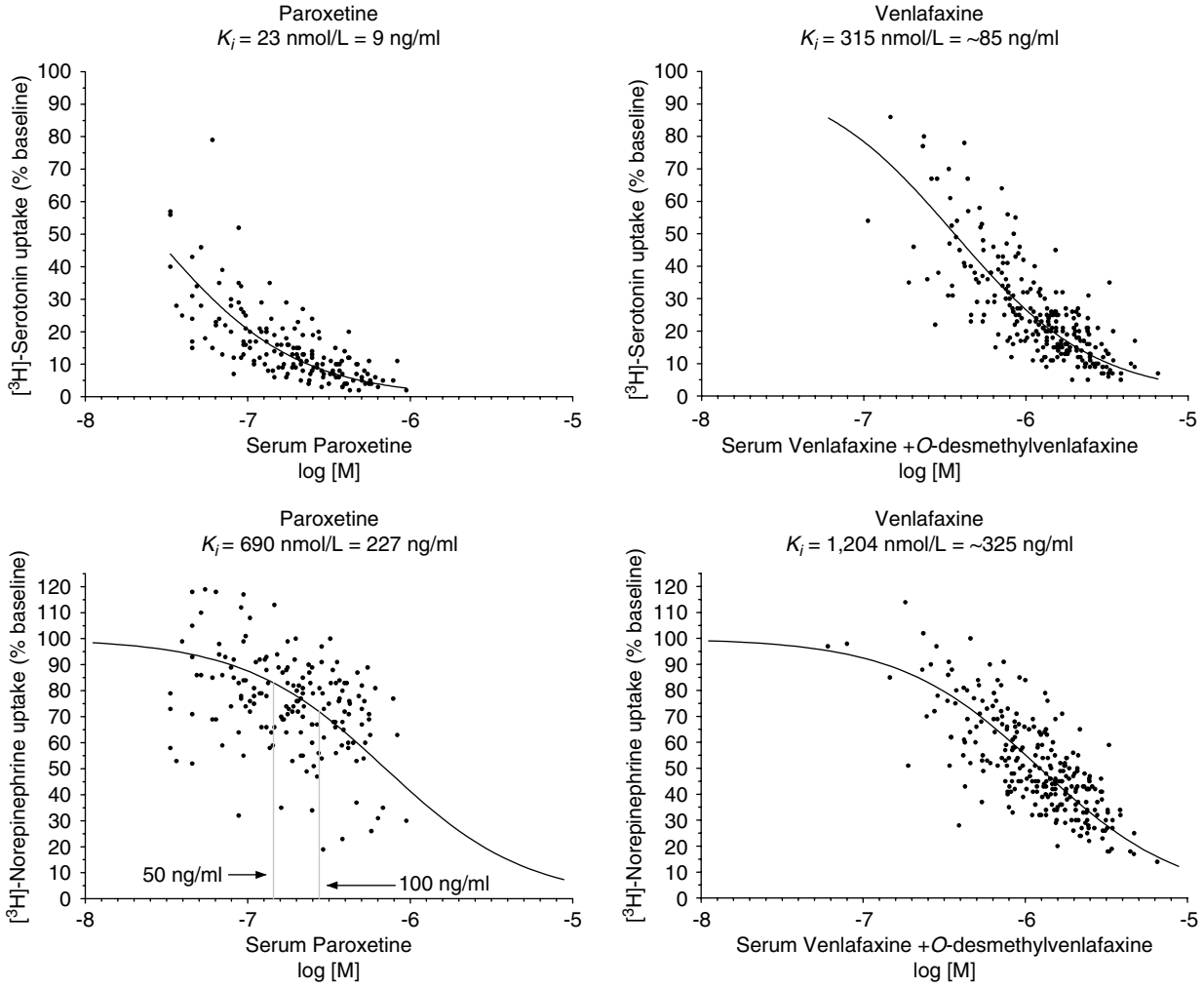

Figure 3 Relationship between SERT and NET occupancy and serum concentrations of paroxetine and venlafaxine + *O*-desmethylvenlafaxine (ODV) in patients with major depressive disorder. Curves were generated using all data points in which accurately quantifiable medication was present in serum. Venlafaxine and ODV possess identical affinities for the SERT and NET and are combined to give a total active molar concentration in serum. The molecular weights of venlafaxine and ODV are 277 and 263, respectively. We added the individual molar concentrations of each drug together to provide a total molar concentration but used a value of 270 to convert combined molar concentrations of these two medications into a ng/ml estimate of the K_i value. Classic one-site competition curves used to describe drug-transporter interactions resulted in goodness of fit (r^2) and 95% confidence intervals (CI) of: paroxetine at the SERT ($r^2 = 0.49$, 95% CI = 21–25 nmol/l), venlafaxine at the SERT ($r^2 = 0.62$, 95% CI = 294–338 nmol/l), paroxetine at the NET ($r^2 = 0.08$, 95% CI = 590–805 nmol/l) and venlafaxine at the NET ($r^2 = 0.54$, 95% CI = 1126–1288 nmol/l). For comparison, regression analysis of serum paroxetine concentrations of 25, 50, and 100 ng/ml correspond with 94, 89, and 80% of baseline uptake or 6, 11, and 20% NET occupancy, respectively. Log [mol/l] serum concentrations of paroxetine = log ([ng/ml]/329 400 000). Log [mol/l] serum concentrations of venlafaxine = log ([ng/ml]/277 400 000). Log [mol/l] serum concentrations of ODV = log ([ng/ml]/263 400 000).

widely believed that cerebrospinal fluid (CSF) concentrations of drug represent an accurate estimate of drug concentrations in the extracellular matrix surrounding neurons. However, there is surprisingly little data available regarding CSF concentrations of new generation antidepressants as a function of drug dosage or serum concentrations. Of the studies that exist, several have shown a strong correlation between CSF and serum concentrations of several other classes of psychopharmacological agents. For example, a nearly perfect correlation between CSF concentrations and predicted 'free' serum concentrations exists for imipramine and desipramine (Muscettola *et al*, 1978). There is also a significant correlation for other tricyclic antidepressants (Hanin *et al*, 1985; Potter *et al*, 1985) and the typical antipsychotic drug haloperidol (Forsman and Ohman, 1997; Linkowski *et al*, 1984). Similar findings are present, albeit with much smaller data sets, for SSRIs including fluoxetine (Martensson *et al*, 1989) and paroxetine (Lundmark *et al*, 1994). These data, and others (Rochat *et al*, 1999; Mahar-Doan *et al*, 2002; Weiss *et al*, 2003), suggest that active transport processes do not play any meaningful role.

Measurement of drug concentrations in serum typically reveals only total drug concentration. Although a value representing percent protein bound is available for most drugs, it is apparent that pharmacogenetics, as well as differences in assay technique, result in ranges of protein binding rather than an absolute value. In addition, the same dose of drug can result in widely differing serum concentrations between individuals. Indeed, multi-fold differences

Table 2 Analysis Results: Secondary Clinical Efficacy Parameters at week 8 LOCF Endpoint (ITT Population)

	Paroxetine CR		Venlafaxine XR		Paroxetine CR		Venlafaxine XR				
Efficacy parameters	***N***	**Baseline score, mean (SD)**	***N***	**Baseline score, mean (SD)**	***N***	**Adjusted mean change from baseline (SE)**	***N***	**Adjusted mean change from baseline (SE)**	**Adjusted mean difference**	**95% CI**	***p*-value**
MADRS total score	40	28.6 (5.05)	41	28.6 (5.97)	37[a]	−16.7 (8.59)	38[a]	−17.3 (8.99)	0.60	−3.42, 4.54	0.784
						Number (%) of responders		Number (%) of responders			
CGI-I responders		N/A		N/A	40	27 (67.5%)	38[a]	30 (78.9%)		−30.9, 8.00	0.312
		Baseline score, mean (range)		Baseline score, mean (range)		Mean change from baseline (range)		Mean change from baseline (range)	Mean difference		
CGI-S	40	4.4 (3–6)	41	4.6 (4–6)	40	−1.7 (−5 to 0)	38[a]	−2.0 (−5 to 0)	−0.3	−0.259, 0.962	0.263

Post hoc *MADRS efficacy analyses*

Response		*n* (%) experiencing ⩾50% decrease in total score			χ^{2}[b]	*p*-value
LOCF	37[a]	24 (64.9%)	38[a]	27 (71.1%)	0.330	0.626
Completers	32	24 (75.0%)	32	24 (75.0%)	0.005	1.000
Remission		*n* (%) with ⩽10 total score			χ^{2}[b]	*p*-value
LOCF	37[a]	17 (45.9%)	38[a]	24 (63.2%)	2.252	0.167
Completers	32	17 (53.1%)	32	23 (71.9%)	2.419	0.196

MADRS: Montgomery–Asberg Depression Rating Scale; CGI-I: Clinical Global Impression global improvement; CGI-I responders: patients with a score of 1 (very much improved) or 2 (much improved), CGI-S: Clinical Global Impression severity of illness; LOCF: Last Observation Carried Forward.
[a]Eleven patients (5 PAR+6 VEN) discontinued from the study before their first scheduled MADRS assessment at week 2, and therefore are not included in the analyses. Seventy-five patients comprise the MADRS evaluable population.
[b]For significance at the 0.05 level, χ^{2} should be greater than or equal 3.84. No distribution was significant; no comparisons $p<0.05$ using two-tailed Fisher's Exact Test.

in paroxetine and venlafaxine serum concentrations are observed in patients receiving identical doses (Figure 1). Although differences in body mass may play a role in these variances, inter-individual pharmacogenetic differences in genes controlling all aspects of drug availability and kinetics are also important. Thus, simply measuring serum drug concentrations and calculating transporter occupancy based upon reported mean percentage of protein binding and *in vitro* transporter affinity is clearly not an acceptable method. Indeed, we speculate that a 'therapeutic window' for antidepressants may finally be found if transporter occupancy becomes the important variable rather than serum concentrations.

We have circumvented these confounds by measuring transporter occupancy directly in individual patient serum samples. Because free drug concentrations in serum approximate free drug concentrations in CNS extracellular fluid, we have modified standard *in vitro* assays for monoamine uptake to closely approximate the conditions *in vivo*. Rather than neurons being exposed to drug in extracellular fluid, we exposed cell lines expressing either the human SERT or NET directly to human serum containing antidepressants that inhibit SERT and NET function. Serum exhibits the necessary characteristics of osmolarity, glucose, and buffering capacity to sustain active transport. Because the serum is essentially unadulterated (ie, no additional buffers, chemicals, and procedures etc.), the important bound : free ratio is maintained (Gilmor *et al*, 2002; Davidson *et al*, 2005). All subjects serve as their own control and therefore, a sample from each subject at baseline (ie, drug-free) serves as 0% occupancy. Full validation of this assay will require concomitant assessment of transporter occupancy using the *ex vivo* assay used here and positron emission tomography (PET) imaging which remains the standard for *in vivo* occupancy data. Such studies are now underway in our laboratory.

As a whole, and as expected, each medication produced dose-dependent increases in SERT and NET inhibition with mean maximal SERT inhibition of ⩾85% and mean maximal NET inhibition of 36 and 60% for paroxetine and venlafaxine, respectively, at week 8 (Figure 2). These data should be interpreted with the understanding that at each dose listed in Figure 2, there are wide variations in drug concentrations and, therefore, SERT and NET inhibition. The maximal NET inhibition differed between paroxetine and venlafaxine, but this must also be interpreted with the knowledge of the maximal doses chosen for the end of the study (ie, 75 mg/day paroxetine CR and 375 mg/day venlafaxine XR). Higher or lower final doses for either medication would have altered the maximal NET

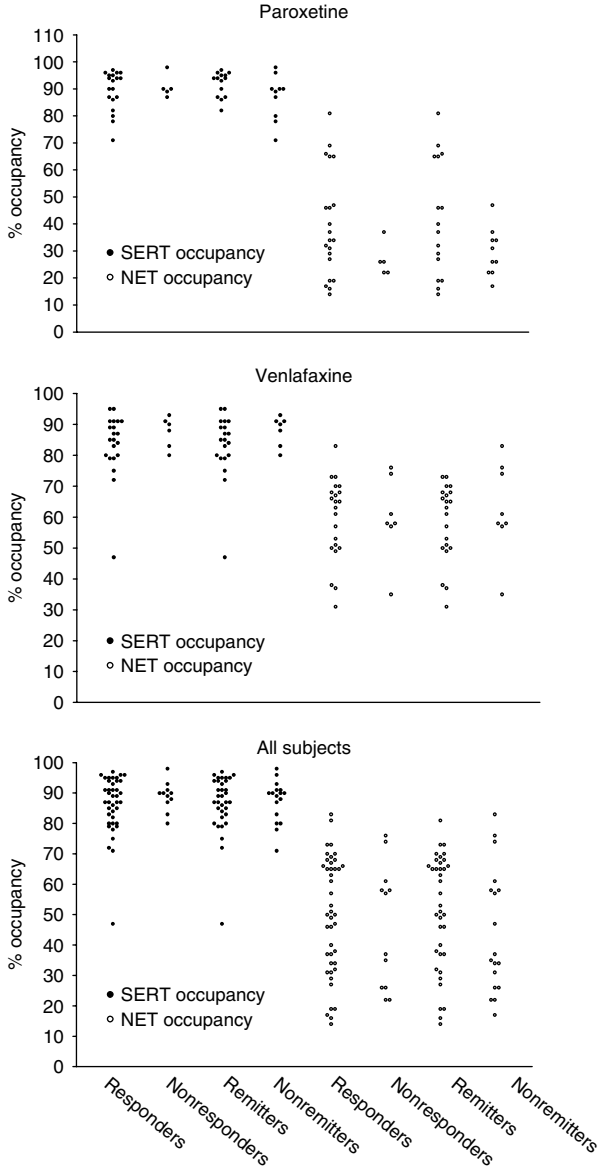

Figure 4 Relationship between treatment response and SERT or NET occupancy at week 8. *Y* axis is percentage occupancy of the relevant transporter (see SERT and NET designations at bottom of graph). All remitters are also shown as data points under responders and all nonresponders are also shown as data points under nonremitters. Data is only used from patients who completed the entire 8 week protocol and were able to provide serum samples for occupancy estimation at week 8 ($N = 24$ for paroxetine SERT and $N = 26$ for paroxetine NET; $N = 27$ for venlafaxine SERT, and $N = 29$ for venlafaxine NET). The bottom panel does not distinguish between medications but only illustrates response and transporter occupancy. No significant interactions were detected (two-way ANOVA).

inhibition. The replication of our previous findings (Gilmor *et al*, 2002; Davidson *et al*, 2005) showing partial NET inhibition with paroxetine provides some rationale for the finding that a 40 mg/day dose of paroxetine was found to be more efficacious in preventing depressive recurrences than a 20 mg/day dose in patients previously treated effectively with a 40 mg/day dose (Franchini *et al*, 1998). In addition, there is evidence that paroxetine is an effective treatment for refractory depression at higher doses of 30–40 mg/day at which NET inhibition is likely to begin (Tyrer *et al*, 1987).

Direct comparisons of venlafaxine and paroxetine appeared to favor venlafaxine (Poirier and Boyer, 1999; Ballus *et al*, 2000). However, as has been pointed out elsewhere (Nemeroff *et al*, 2008), in these studies low doses of paroxetine were often compared to high doses of venlafaxine, and serum paroxetine concentrations were not reported when doses of 30–40 mg/day were used. In effect, therefore, these studies have compared only the SSRI properties of low doses or probably low serum paroxetine concentrations to venlafaxine's dual transporter inhibition at moderate to high doses in some individuals.

Plotting of all data points based on known serum concentrations in each individual sample and transporter inhibition yields classic drug-receptor competition curves (Figure 3). The data in Figure 3 show that the majority of data points reveal that SERT inhibition is approaching or greater than 80% (20% of baseline along *Y* axis), which appears to be a threshold for efficacy (Meyer *et al*, 2001, 2004). These data verify that both drugs can be effective (ie, provide 80% SERT inhibition) even at low doses in some individuals.

These data also show that neither of the drugs produce the magnitude of NET inhibition that they provide of SERT inhibition, though both agents again display classic pharmacological binding curves as expected. It is clear that at the serum concentrations obtained in this particular study, venlafaxine XR treatment can produce a greater degree of NET inhibition. At a serum concentration of 100 ng/ml, paroxetine produces an expected (see curve) 22% NET inhibition (ie, 78% of baseline). This serum concentration is well within the serum concentrations observed in this study (Figure 1). As shown in the lower right portion of Figure 3, venlafaxine occupies 50% of the NET at a combined venlafaxine + ODV concentration of approximately 325 ng/ml (−5.92 log [M]). These concentrations appear to be readily attainable. Only a limited number of venlafaxine samples and very few paroxetine samples produced $\geqslant$80% NET inhibition.

There are no available data on how much NET inhibition would be necessary in the face of concurrent $\geqslant$80% SERT inhibition to produce added potential beneficial effect on efficacy. The only estimation of NET inhibition during high-dose venlafaxine treatment showed only a partial inhibition of the NET, as assessed with measures of blood pressure in response to challenge with the false transmitter tyramine (Harvey *et al*, 2000). These investigators observed that neither venlafaxine (75 or 375 mg/day) nor sertraline were significantly different than baseline; however, the 375 mg dose clearly showed a trend portending partial NET inhibition. In that study, only maprotiline produced robust and clear evidence of NET inhibition. Recently, Blier and colleagues (Debonnel *et al*, 2006) reported similar findings in depressed subjects receiving venlafaxine. Therefore, if the clinical literature suggesting some added benefit of the NET inhibiting component of venlafaxine is correct, partial NET inhibition (in the face of ongoing $\geqslant$80% SERT inhibition) may be sufficient and both medications studied here can provide partial NET inhibition. No other SSRIs are predicted to possess NET inhibition at any reasonable dose. The reader should note that these studies utilizing the tyramine pressor test are a peripheral measure of putative NET inhibition on sympathetic nerve terminals. It remains

to be definitively established whether this accurately reflects CNS NET inhibition.

In this study both paroxetine CR and venlafaxine XR were efficacious and generally well tolerated. The mean change from baseline in MADRS total score at endpoint was approximately 17 points in both treatment groups, which represents a substantial degree of symptom reduction. To put this level of MADRS change in clinical perspective using other efficacy endpoints, the mean proportion of patients considered to be either much improved or very much improved at endpoint based on the CGI was approximately 70% for paroxetine and 80% for venlafaxine. Furthermore, the proportion of patients considered to be in remission (ie, essentially symptom free) at endpoint, based on achieving a MADRS total score $\leqslant 10$, was 46% for paroxetine and 63% for venlafaxine, again reflective of a substantial treatment response; however, without a placebo control group, these efficacy data must be interpreted cautiously. None of the differences between paroxetine and venlafaxine on any of the efficacy parameters were statistically significant; however, again this study was not appropriately designed or powered to definitively compare the efficacy of these two agents.

Although not a primary design of the study, no differences were observed between the magnitude of NET inhibition and patient response in subjects who completed the study; however, it was of interest that all five of the paroxetine-treated subjects who had $>60\%$ NET inhibition attained remission (Figure 4). The value of this observation is unclear as it was not fully replicated in the venlafaxine-treated subjects.

As noted earlier, whether it be synaptic and extrasynaptic concentrations of serotonin or norepinephrine or clinical response, there must be a relationship between occupancy and biological response. A lack of overt differences between SERT and/or NET occupancy and clinical response in these data should not dissuade the reader from agreeing with this hypothesis. We believe that with the tools currently available, response cannot be correlated to occupancy in the relatively narrow range of occupancies typically observed at therapeutic dosages (ie, 70–90%). We are unaware of detailed studies of the SERT or NET that clearly show the direct relationship between occupancy and changes in available transmitter. This relationship should exist as a classic occupancy–response curve, but it may take a considerable amount of occupancy before monoamine concentrations are altered (ie, monoamine concentrations do not begin to rise until significant occupancy has occurred). Assuming that it is indeed alterations in synaptic monoamines that are responsible for initiating the clinical response process, the myriad of potential downstream processes that ultimately lead to clinical response may further dilute the ability to clearly correlate SERT or NET occupancy with treatment response.

Previous *in vitro* binding data and *in vivo* animal and human studies suggested to us that paroxetine and venlafaxine can produce both SERT and NET inhibition to varying degrees at clinically utilized doses. The present data confirm and extend these findings and provide additional impetus for further research into accurate assessment of the magnitude of combined SERT and NET inhibition and its relationship to treatment response.

In the continued absence of direct measures of NET occupancy *in vivo* in depressed patients (eg, use of ligands in PET or single photon emission computed tomography), this novel *ex vivo* uptake assay may accurately estimate CNS transporter occupancy. Our results support previous *in vitro* and *in vivo* studies that suggest paroxetine, a potent SSRI, inhibits the NET and is a dual uptake inhibitor at high therapeutic serum concentrations. The magnitude of NET blockade does not appear to match that possible with venlafaxine within the doses studied. Thus, classification of drugs based exclusively on their binding profile *in vitro* needs to be interpreted with knowledge about what concentrations are ultimately available at the target site. Finally, whether added NET inhibition, and of what magnitude, to SSRIs results in increased efficacy or decreased latency to efficacy remains to be conclusively determined.

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DISCLOSURE/CONFLICT OF INTEREST

Dr Owens: In the past 3 years, Dr Owens has had research grants from Eli Lilly, Pfizer, GlaxoSmithKline, Merck, Lundbeck, Cyberonics, and Johnson & Johnson. He has consulted to Pfizer, Lundbeck, Sepracor, Johnson & Johnson, Sanofi-Aventis, Forest Labs and received speaker's honoraria from GlaxoSmithKline. Dr Owens has a patent entitled 'A method to estimate transporter occupancy'.

Mr Krulewicz and Dr Carpenter are employees of GlaxoSmithKline and own company stock.

Jeffrey S Simon, M.D. is a member of the GlaxoSmithKline and Wyeth Pharmaceuticals Speaker Bureaus. He has had clinical trial grant support from GlaxoSmithKline, Wyeth Research, Pfizer Pharmaceutical, Bristol Meyer Squibb, AstraZeneca, Eli Lilly, Cephalon, Sanofi, Organon, Sandoz, Merck, Upjohn Pharmacia, Otsuka, Synthelabo, and Forest Laboratories. Dr Simon has also served on advisory boards to Pfizer, Bristol Myers Squibb, Wyeth, Cephalon, Abbott and Tap Pharmaceuticals.

David V. Sheehan has had research support from Abbott Laboratories, American Medical Association, Anclote Foundation, Astra-Zeneca, Bristol-MyersSquibb, Cephalon, Eisai America, Inc., Eli Lilly & Company, Forest Laboratories, GlaxoSmithKline, International Clinical Research (ICR), Janssen Pharmaceutica, Jazz Pharmaceuticals, Kali Duphar Laboratories Inc., Medicinova, Merck, NIH, Novartis Pharmaceuticals Corp., Pfizer, Quintiles, Sanofi-Aventis, Tampa General Hospital, TAP Pharmaceuticals, Worldwide Clinical Trials, and Wyeth-Ayerst Pharmaceutical

Co. Dr Sheehan is a stock shareholder in Layton Bioscience and Medical Outcome Systems. Dr Sheehan received speakers honoraria from Abbott Laboratories, AstraZeneca, Boots Pharmaceuticals, Bristol-MyersSquibb, Charter Hospitals, Dista Products Company, Eli Lilly & Co., Excerpta Medica Asia, GlaxoSmithKline, Hospital Corporation of America, Human, ICI, Janssen Pharmaceutica, Kali-Duphar, Merck, Organon, Novo Nordisk, Pfizer Inc., Solvay Pharmaceuticals, TAP Pharmaceuticals TGH-University Psychiatry Center, and Wyeth-Ayerst Laboratories. In the past 3 years, Dr Sheehan has served as a consultant to Solvay Pharmaceuticals, Avera Pharmaceuticals, MediciNova, Jazz Pharmaceuticals, Roche, Cephalon, GlaxoSmithKline, Sanofi-Synthelabo Research, AstraZeneca, Forest Laboratories, Pierre Fabre, Alza Pharmaceuticals, Bristol-MyersSquibb, Pfizer, National Anxiety foundation, Zars Pharma, Eisai, and Applied Health Outcomes/xCENDA.

Michael E Thase, M.D. serves on the Advisory Boards for AstraZeneca; Bristol-Myers Squibb Company; Cephalon Inc.; Cyberonics Inc.; Eli Lilly & Co.; GlaxoSmithKline; Janssen Pharmaceutica; MedAvante Inc.; Neuronetics, Inc.; Novartis; Organon Inc.; Sepracor Inc.; Shire US Inc.; Supernus Pharmaceuticals; Wyeth Pharmaceuticals, and is a member of the Speakers Bureaus for AstraZeneca; Bristol-Myers Squibb Company; Cyberonics Inc.; Eli Lilly & Co.; GlaxoSmithKline; Organon Inc.; Sanofi Aventis; Wyeth Pharmaceuticals. Dr Thase currently has equity holdings in MedAvante Inc., and receives royalty income from the American Psychiatric Publishing Inc., Guilford Publications, and Herald House. Dr Thase has no clinical trial grant support from the pharmaceutical industry.

Dr Nemeroff: In the past 3 years, Dr Nemeroff consulted to, served on the Speakers' Bureau and/or Board of Directors, has been a grant recipient, and/or owned equity in one or more of the following: Abbott Laboratories, Acadia Pharmaceuticals, American Foundation for Suicide Prevention (AFSP), American Psychiatric Institute for Research and Educations (APIRE), AstraZeneca, BMC-JR LLC, Bristol-Myers-Squibb, CeNeRx, Corcept, Cypress Biosciences, Cyberonics, Eli Lilly & Co., Entrepreneur's Fund, Forest Laboratories, George West Mental Health Foundation, GlaxoSmithKline, i3 DLN, Janssen Pharmaceutica, Lundbeck, National Alliance for Research on Schizophrenia and Depression (NARSAD), Neuronetics, NIMH, NFMH, NovaDel Pharma, Otsuka, Pfizer Pharmaceuticals, Quintiles, Reevax, UCB Pharma, and Wyeth-Ayerst.

Currently, Dr Nemeroff serves on the Scientific Advisory Board for Astra-Zeneca, Johnson& Johnson, Forest Laboratories, Quintiles, PharmaNeuroBoost and NARSAD. He is a grant recipient from NIH, NARSAD, and AFSP. He serves on the Board of Directors of AFSP, APIRE, NovaDel Pharmaceuticals, and the George West Mental Health Foundation. He owns equity in CeNeRx and Reevax. He owns stock or stock options in Corcept and NovaDel. Patents: 'A method to estimate transporter occupancy'; 'transdermal delivery of lithium'.

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