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ORIGINAL ARTICLE

HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition

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The hypothalamic–pituitary–adrenal (HPA) axis has been implicated in the pathophysiology of a variety of mood and cognitive disorders. Neuroendocrine studies have demonstrated HPA axis overactivity in major depression, a relationship of HPA axis activity to cognitive performance and a potential role of HPA axis genetic variation in cognition. The present study investigated the simultaneous roles HPA axis activity, clinical symptomatology and HPA genetic variation play in cognitive performance. Patients with major depression with psychotic major depression (PMD) and with nonpsychotic major depression (NPMD) and healthy controls (HC) were studied. All participants underwent a diagnostic interview and psychiatric ratings, a comprehensive neuropsychological battery, overnight hourly blood sampling for cortisol and genetic assessment. Cognitive performance differed as a function of depression subtype. Across all subjects, cognitive performance was negatively correlated with higher cortisol, and PMD patients had higher cortisol than did NPMDs and HCs. Cortisol, clinical symptoms and variation in genes, NR3C1 (glucocorticoid receptor; GR) and NR3C2 (mineralocorticoid receptor; MR) that encode for GRs and MRs, predicted cognitive performance. Beyond the effects of cortisol, demographics and clinical symptoms, NR3C1 variation predicted attention and working memory, whereas NR3C2 polymorphisms predicted memory performance. These findings parallel the distribution of GR and MR in primate brain and their putative roles in specific cognitive tasks. HPA axis genetic variation and activity were important predictors of cognition across the entire sample of depressed subjects and HR. GR and MR genetic variation predicted unique cognitive functions, beyond the influence of cortisol and clinical symptoms. GR genetic variation was implicated in attention and working memory, whereas MR was implicated in verbal memory.

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INTRODUCTION

The stress-responsive hypothalamic–pituitary–adrenal (HPA) axis has been implicated in the pathophysiology of anxiety and depression as well as cognitive functioning. The axis consists of stimulating forward and feedback inhibition loops involving the brain, pituitary and adrenal glands, which regulates glucocorticoid production. Cortisol released from the adrenal glands, binds in brain with high affinity to mineralocorticoid receptors (MRs) and with lower affinity to glucocorticoid receptors (GRs). GR is distributed widely throughout the primate brain, whereas MR is heavily localized to the hippocampus.[1] In addition, glucorticoid responsive elements are found in the regulatory regions of many genes in brain. Cortisol exerts its tonic influences predominantly via hippocampal MR, whereas feedback actions at the level of the pituitary and activated brain areas such as the amygdala are mediated by GR.[2,3] The development of major depression has been postulated to reflect a dysregulation of MR and/or GR within the HPA system.[4,5]

Upwards of 40–60% of depressed patients experience hypercortisolemia[6] or other disturbances of the HPA system, such as flattened circadian state rhythm,[7] or an earlier[8] or elevated nadir.[9] However, we and others have found that elevated HPA activity is more closely associated with specific depression subtypes, such as psychotic features. Psychotic major depression (PMD) patients demonstrate elevated activity of the HPA axis as compared with nonpsychotic depressives (NPMD) or healthy controls (HC).[10–13] PMD patients have significantly elevated evening[11] and afternoon (1300–1600 hrs)[10] serum cortisol levels. Furthermore, PMD patients demonstrate a blunted response to fludrocortisone, a mixed mineralocorticoid/glucocorticoid agonist,[12,14] high rates of non-suppression on challenge with dexamethasone, with particularly high post-dexamethasone cortisol levels.[13]

Disrupted cognition is also a feature of depression with the most consistent deficits being in memory and executive function.[15] PMD patients have even greater decrements in cognition than do NPMDs.[16] Furthermore, higher cortisol levels have been associated with impaired cognitive functioning in both HC and depressed patients.[16,17]

The relative roles of GR and MR in cognitive dysfunction in depression have been a focus of limited study, although, animal data have long pointed to a role for MR in both cortisol secretion and memory and executive function performance.[18] In HCs, blocking MR impairs memory and executive function.[19] In contrast, fludrocortisone decreases cortisol in HC and depressives and enhances verbal memory;[20] significant correlations between cortisol inhibition and verbal memory (list learning) performance were observed. MR stimulation with fludrocortisone inhibits cortisol secretion,[21] but fludrocortisone inhibition of cortisol is attenuated in patients with PMD suggesting impaired MR function in those patients.[12] Last, there is evidence for decreased MR expression in the hippocampus and prefrontal cortex in depressed patients.[22,23]

Although MRs mediate neuronal changes required for learning and memory, *NR3C2* (MR) genetic variation thus far has not been

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associated with specific aspects of cognition. *NR3C2* genetic variation has been associated with higher serum levels of brain-derived neurotrophic factor, where those with intermediate and high exposure to physical neglect showed higher serum levels of brain-derived neurotrophic factor but only in those with the CC genotype.[24] Although APOE gene is confirmed as a high risk of Alzheimer's disease (AD), Sun *et al.*[25] examined four large data sets, looking for other potential risk genes. They concluded that one of 10 potential risk genes was *NR3C2* (MR). As MR is densely distributed in the hippocampal region[26] and involved in hemodynamic centers in brain,[27] it may be involved in the progression of AD in response to stressful stimuli.[28]

Recent studies on specific single-nucleotide polymorphisms (SNPs) for GR indicate that some are associated with altered GR sensitivity,[29] and in conjunction with COMT genetic variation are associated with poorer working memory in HC.[30] In addition, Young's group reported that the GR antagonist mifepristone significantly improves visual memory in bipolar depression.[31] Our group has recently reported that GR (but not MR) genetic variation accounted for a significant amount of variance in mean cortisol levels and severity of psychosis.[32] However, the relationship of variation in NR3C1 that encodes for GR gene and the directionality of influence on cognition in depression is not yet known.

We have reported previously on potential relationships of cortisol and cognition in limited samples of subjects,[16] but neither we nor others have explored the relationships among genetic variation, clinical symptoms and cognition. The specific aim of this project was to extend on previous findings on cortisol dysregulation to cognitive performance by exploring whether HPA axis genetic variation (see Table 1 for list of SNPs) also contributes to specific cognitive performance beyond that predicted by cortisol or clinical measures.

MATERIALS AND METHODS

Participants

Psychiatric participants were recruited through inpatient and outpatients facilities at Stanford University or self-referred from online and print study advertisements. Fifty-nine patients with PMD and 58 patients with NPMD participated in two waves of a larger study on HPA axis in depression.

Depressed patients were required to have a minimum score of 21 on the 21-item Hamilton Depression Rating Scale and a minimum score of 6 on the Thase Core Endogenomorphic Scale, with one exception of a PMD who scored 5. These latter two criteria were designed to ensure inclusion of participants with similar minimum levels of severity of endogenous-type symptoms. PMDs were also required to have a minimum total score of 5 on the positive symptom subscale of the Brief Psychiatric Rating Scale, which consists of four items: conceptual disorganization, suspiciousness, hallucinations and unusual thought content. A score of 4 on the Positive Symptom Subscale indicates no positive psychotic symptoms. NPMD subjects had no history of psychotic symptoms. All patients met the Diagnostic and Statistical Manual of Mental Disorders criteria for a current major depressive episode, with or without psychotic features.

Healthy control subjects were recruited through online and print study advertisements. Overall, 63 HC participated in the larger HPA study and 29 provided blood samples for genetic analyses. HC were assessed for Axis I disorders with the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders. They had a score of <6 on the 21-item Hamilton Depression Rating Scale and no psychotic symptoms as measured by the Brief Psychiatric Rating Scale positive symptom subscale. Furthermore, they had no current or history of Axis I psychiatric illness.

Participants were allowed to remain on their psychiatric medications but were required to maintain a stable medication dosing regimen for at least

Table 1. List of *NR3C1* (GR) and *NR3C2* (MR) SNPs examined in this study

Gene	*Function*	*Brain location in humans*	*SNPs studied*
HPA axis genes assessed			
NR3C1 (GR)	Feedback inhibition of HPA axis; cognition; immune response	Cortex widely, hypothalamus, amygdala, hippocampus	$N=10$ [a]rs56149945 (formerly rs6195) [b]rs6198 rs33388 [c]rs2918419 rs10052957 rs10482633 [b]rs12521436 [b]rs12655166 [b]rs17209258 rs41423247
NR3C2 (MR)	Inhibitory control of HPA axis; memory; blood pressure	Hypothalamus, hippocampus, amygdala	$N=13$ [b]rs5525 [a]rs5530 rs1879829 rs2070951 [b]rs2272089 rs3910052 rs4835488 rs6535578 rs7658048 [b]rs7694064 [b]rs10213471 [a]rs17024360 [b]rs17484245 rs2070950 [b]rs5522
FKBP5	Co-chaperone to heat shock protein for GR; stabilizes GR confirmation	See GR	$N=2$ rs1360780 rs3800373

Abbreviations: FKBP5, FK506-binding protein 5; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; MR, mineralocorticoid receptor; SNP, single-nucleotide polymorphism. [a]Little or no variance in the SNPs; SNP not used in analyses. [b]Less than four total in the rare homozygous, collapsed into the heterozygous SNP group. [c]Only two SNP variations present.

Table 2. Subject demographics

	PMD (N = *46)*	*NPMD* (N = *37)*	*HC* (N = *46)*	*Analysis*	Post hoc *comparison*
Age	36.67 (12.4)	42.38 (12.4)	36.17 (12.6)	F(2,126) = 3.01, $P = 0.053$	
Education	15.04 (2.8)	14.81 (1.9)	15.68 (2.2)	F(2,126) = 1.58, ns	
WTAR predicted FIQ	*N* = 35 110.57 (10.5)	*N* = 32 109.16 (10.4)	*N* = 42 111.31 (9.0)	F(2,106) = 0.433, ns	
Gender				*$\chi^2 = 0.454$, ns*	
Male	21	15	22		
Female	25	22	24		
Ethnicity				*$\chi^2(8) = 8.51$, ns*	
Caucasian	31	28	26		
African Am.	4	3	3		
Asian Am.	5	1	10		
Latino	2	4	3		
Other	1	1	1		
Daily psych medications[a]	*39/46*	*18/37*	*0/46*	*$\chi^2(2) = 67.46$, $P < 0.001$*	
Antidepressants	32/46	17/37			
Antipsychotics	28/46	2/37			
Anxiolytics/benzodiazapines	19/46	4/37			
Mood stabilizers	8/46	3/37			
Daily psych medications–genetic sample only[a]	*29/33*	*11/23*	*0/24*	*$\chi^2(1) = 10.64$, $P = 0.001$*	
Antidepressants	24/33	10/23		$\chi^2(1) = 4.96$, $P = 0.027$	
Antipsychotics	21/33	2/23		$\chi^2(1) = 16.90$, $P < 0.001$	
Anxiolytics/benzodiazapines	15/33	3/23		$\chi^2(1) = 6.53$, $P = 0.011$	
Mood stabilizers	7/33	2/23		$\chi^2(1) = 1.54$, ns	
Comorbidity diagnoses					
Panic disorder	9	3			
Agorophobia	3	2			
Social Phobia	7	4			
PTSD	5	5			
GAD	5	3			
HDRS	30.33 (5.4)	24.08 (3.2)	0.56 (93)	F(2,126) = 811.5, $P < 0.001$	*P* > (*N* > HC)
Thase Endogenous Scale	9.32 (1.8)	8.21 (1.7)	0.087 (0.28)	F(2, 126) = 576.6, $P < 0.001$	*P* > (*N* > HC)
BPRS	48.04 (7.4)	33.62 (4.2)	18.56 (1.1)	F(2, 126) = 398.5, $P < 0.001$	*P* > (*N* > HC)
Positive symptom scale	12.04 (3.8)	4.21 (.53)	4.07 (.33)	F(2, 126) = 177.7, $P < 0.001$	*P* > (*N* = HC)

Abbreviations: BPRS, Brief Psychiatric Rating Scale; FIQ, Full-scale Intelligence Quotient; GAD, Generalized Anxiety Disorder; HC, healthy controls; HDRS, Hamilton Depression Rating Scale; NPMD, nonpsychotic major depression; PMD, psychotic major depression; PTSD, post-traumatic stress disorder; WTAR, Wechsler Test of Adult Reading. Under *post hoc* comparisons, *P*, psychotic major depression; *N*, nonpsychotic major depression. This refers to the significance (*P*-value) of the pairwise comparisons of the three groups. For example, under HDRS, PMD subjects are significantly higher than both NPMDs and HCs, and NPMDs are significantly higher than HCs. [a]Any daily psychiatric medication includes use of antidepressants, antipsychotics, anxiolytics or mood stabilizers.

1 week prior to the start of the study. Depressed patients were taking a combination of antidepressants, antipsychotics, anxiolytics and mood stabilizers (see Table 2).

Of the 153 original subjects, various subsets of patients are used in different analyses. See Supplementary Figure 1 for a consort chart of usable subjects in each aspect of the omnibus study. Nine patients had unusable or missing neuropsychological assessments, and 15 additional participants were taking estrogens, so the total sample for neuropsychological analyses is 129. Nine patients had the CVLT-1 instead of the CVLT-II, so those patients were dropped only from the CVLT analyses. An additional 6 had missing or unusable cortisol data, and 44 were missing genetic data. Thus, for the genetic analyses the sample was 80 subjects. The sample sizes were based on effect sizes of our previous work on cortisol and neuropsychological testing results.

Procedure

The study was approved by the Stanford University Institutional Review Board, and all subjects gave written informed consent before screening. Eligibility screening procedures included the SCID, Hamilton Depression Rating Scale, Brief Psychiatric Rating Scale, clinical laboratory tests, comprehensive metabolic panel, urine drug screening and urine screening for pregnancy in female subjects. If participants met inclusion criteria at eligibility screening, they returned for baseline procedures.

At baseline, participants were re-administered the Hamilton Depression Rating Scale-21 and the Brief Psychiatric Rating Scale to assess for clinical symptomatology. Participants then were assessed for cognitive function. Overnight blood sampling then took place on the Stanford Hospital Clinical and Translational Research Unit. An intravenous line was inserted at 1600 hrs and blood collected at the top of each hour for cortisol from 1800 to 0900 hrs the next day. Sixteen blood samples per patient were collected. Part way through the first wave of study, collection of blood samples for genetic analysis began. The majority of available subjects consented to provide blood for genetic analyses.

Data on subsets of participants have been previously reported. Cortisol and cortisol/cognitive data were previously reported[11] on a subset of 73 of 129 subjects reported here. Data on another subset 78 of 80 subjects who had both cortisol and genetic data were also recently reported.[32] No published paper from this data set has examined the relationships of cognition, cortisol and genetics together.

Cognition

Participants completed a neuropsychological assessment battery that assessed four primary cognitive domains: attention, working memory, executive function and verbal memory (see ref. 16 for full details and Table 3 for a list of tests).

Cortisol determination

Cortisol assays were conducted by the Brigham Women's Hospital, General Clinical Research Laboratory in Boston. The analytic sensitivity for cortisol was 0.4 mcg dl^{-1} with a coefficient of variation of $< 7.9\%$. Because of the natural diurnal rhythm of the cortisol slopes, the 15-h blood collection period was divided into two phases based on the apparent nadir: the evening level from 1800 to 0100 h and the morning level from 0100 to 0900 h. These epochs correspond to the natural descending and ascending slopes of the cortisol rhythm and are based on the nadir observed in previous studies[11] of subsamples from this study. Cortisol means were then computed for these two epochs (1800–0100 and 0100–0900 hrs).

Medication. Patients were taking a variety of psychiatric medications: antidepressants, anxiolytics, antipsychotics and/or mood stabilizers. The sample size is not large enough to subdivide into specific medications; however, because benzodiazepines in particular can affect cognition (specifically memory) subjects were classified by whether or not they were regularly taking them, and this variable was entered into the regression model.

Genetics. Blood was collected for assessment of HPA axis genetic markers and herein we report on three genes: *NR3C1* (GR), *NR3C2* (MR) and FK506-binding protein 5 (FKBP5). Alleles studied were selected using a standard protocol that utilized 5 specific criteria (see ref. 32 for more details).

DNA was extracted from EDTA-treated whole blood using the Gentra Puregene kit (Qiagen, Valencia, CA, USA). Genotyping was performed using Taqman real-time PCR (Applied Biosystems, Foster City, CA, USA). All genotypes were tested for deviation from Hardy–Weinberg equilibrium. SNPs and their frequencies were assayed for each HPA axis gene.[32] Twenty-seven SNPs in total were assessed (see Table 1 for list of SNPs; Supplementary Figures 2 and 3 for linkage disequilibrium (LD) maps; Supplementary Table 1 for allelic distribution). SNPs that had no or minimal variability in our sample were excluded from the analysis. If SNPs had fewer than four subjects across all three groups homozygous for the rare allele, they were collapsed into the heterozygous group.

Statistical analyses

All analyses were conducted using SPSS statistical software. First, analysis of variance and χ^2 analyses were used to examine group differences in demographic variables (age, education and gender), as well as clinical ratings (21-item Hamilton Depression Rating Scale, Brief Psychiatric Rating Scale). Next, analysis of covariances were utilized to examine differences among the three diagnostic groups for each cognitive test. As age influences neuropsychological functions, it was used as a covariate in the model. Then, analysis of variances were utilized to examine differences in evening and early morning cortisol between the three groups, with age as a covariate, as it is also known to influence cortisol. To account for the number of comparisons, the alpha level was set to $P \leqslant 0.005$ for each omnibus test. On subsequent pairwise comparisons, an alpha level of 0.05 was used.

Finally linear regression analyses were run predicting cognitive performance using subjects across all three groups. To avoid imposing our own bias, we ran a regression model that allowed the data to guide the outcomes, resulting in the most important predictors in the model to be detected. Thus, a forward regression approach was used for each dependent variable, and variable entry was set at 0.05 and variable removal was set at 0.10. The dependent variables were the individual cognitive tests. Independent variables included age, sex, depression severity, psychotic symptom severity, daily benzodiazepines use, mean evening cortisol (1800–0100 hrs), mean early morning cortisol (0100–0900 hrs) and all SNPs in a given gene. We analyzed for effects of *NR3C1* (GR), *NR3C2* (MR) and *FKPB5* separately. The final model which was developed based on the forward regression was set to an overall significance level $P \leqslant 0.005$. This was done to account for the large number of comparisons. Secondary analyses that examined the independent contributions of the chosen variables was set to alpha < 0.05.

Table 3. Means (and s.d.s) of neuropsychological measures and cortisol (mcg dl^{-1}) by group

	PMD (N = *46*)	*NPMD* (N = *37*)	*HC* (N = *46*)	*Analysis and* post hoc *comparisons*
Attention				
Digit span forwards	10.41 (3.0)	10.51 (2.7)	11.32 (2.1)	F(2,118) = 1.38, ns
Trail making test A	34.2 (14.5)	29.75 (12.8)	24.54 (8.8)	F(2,123) = 7.60, $P = 0.001$, $P > (N = H)$
Working memory				
Digit span backwards	6.78 (2.3)	7.71 (2.4)	8.12 (2.5)	F(2,118) = 3.60, $P = 0.03$
Letter number sequencing	9.60 (2.8)	11.30 (2.6)	11.63 (2.9)	F(2,124) = 7.64, $P = 0.001$, $P < (N = HC)$
Executive Function				
Stroop (Color Word)	36.24 (10.2)	42.11 (9.6)	46.80 (12.8)	F(2,124) = 12.23, $P < 0.001$, $P < (N = HC)$
Trail making test B	81.05 (34.8)	68.89 (25.6)	60.63 (24.6)	F(2,121) = 5.79, $P = 0.004$, $P > (N = HC)$
COWA (FAS)	37.30 (11.7)	38.14 (10.6)	45.82 (13.2)	F(2,123) = 6.52, $P = 0.002$, $(P = N) < HC$
Verbal Memory				
CVLT-II	$N = 40$	$N = 33$	$N = 41$	
Total learning trials 1–5	45.00 (12.4)	47.52 (9.3)	56.61 (9.1)	F(2,110) = 12.74, $P < 0.001$, $(P = N) < HC$
Short-delay free	9.45 (3.6)	10.58 (3.5)		
Short-delay cued	10.35 (3.3)	11.73 (2.6)	12.68 (2.4)	F(2,110) = 7.08, $P = 0.001$, $P < (N = HC)$
Long-delay free	9.45 (4.0)	11.03 (3.2)	12.56 (2.8)	F(2,110) = 8.32, $P < 0.001$, $P < (N = HC)$
Long-delay cued	10.30 (3.4)	11.70 (2.6)	12.85 (2.5)	F(2,114) = 7.94, $P = 0.001$, $P < (N = HC)$
Recognition	14.20 (2.2)	14.61 (2.1)	14.85 (1.6)	F(2,114) = 1.17, ns
Logical memory				
Immediate recall	21.80 (6.4)	22.92 (7.6)	28.75 (8.4)	F(2,122) = 10.30, $P < 0.001$, $(P = N) < HC$
Delayed recall	20.44 (8.2)	23.00 (8.1)	29.77 (6.8)	F(2,122) = 16.77, $P < 0.001$, $(P = N) < HC$
Recognition	24.36 (2.9)	25.14 (2.6)	26.98 (2.3)	F(2,121) = 11.25, $P < 0.001$, $(P = N) < HC$
Mean cortisol 1800–0100	$N = 43$ 4.82 (2.4)	$N = 37$ 3.63 (1.6)	$N = 43$ 3.37 (1.3)	F(2,120) = 7.37, $P = 0.001$, $P > (N = HC)$
Mean cortisol 0100–0900	$N = 43$ 9.20 (4.0)	$N = 37$ 9.07 (2.4)	$N = 43$ 8.70 (2.2)	F(2,120) = 0.322, ns

Abbreviations: COWA (FAS), Controlled Oral Word Association Test using the letters F, A, S; CVLT-II, California Verbal Learning Test 2nd Edition. HC, healthy controls; NPMD, nonpsychotic major disorder; PMD, psychotic major depression. Analysis include age as a covariate.

In addition to examining individual SNPs, potential haplotypes were created separately for NR3C1 and NR3C2. NR3C1 potential haplotypes were created using five NR3C1 SNPS, including ER22/23EK (rs6189 and rs6190), rs6195, rs41423247 and rs6198, replicating the methods of Kumsta *et al*.[33] Similar to Kumksta, only Haplotypes 1, 4 and 5 had adequate sample size for further analyses; thus, haplotypes 2 and 3 from the combination of SNPs were not examined. NR3C2 haplotypes were created using rs5522 an rs2070951, which replicates several other studies.[34,35] For all haplotype analyses, if the participant had at least one of each of the target alleles, they were considered a 'potential haplotype carrier'. Regressions were performed as outlined above with the exception that instead of adding the individual SNPs into the model, the potential carrier status of each haplotype (yes/no) was used.

RESULTS

Demographics

Across the three groups, there were no differences in age, education, gender, estimated intelligence quotient, or ethnicity (see Table 2). As expected, there were significant differences in all psychiatric rating scales (Hamilton Depression Rating Scale, Thase Endogenous scale, Brief Psychiatric Rating Scale and Positive Symptom Subscale). PMDs were more severe on all measures compared with NPMD, and both depression groups were higher than the HC. The only exception was the Positive Symptom Subscale, in which NPMD patients and HC did not differ.

Cognitive function

PMD patients performed significant worse than did both HCs on almost all measures of cognition (all *P*'s < 0.005; see Table 3), with the exception of digit span and CVLT-II recognition memory and worse than NPMDs on most measures of attention, working memory and executive function. NPMDs and HCs were generally similar on performance with a few exceptions where NPMDs did more poorly, including COWA, learning on CVLT-II and Logical Memory. Interestingly, on the verbal list learning measure, total learning over the five trials differentiated the three groups, with PMDs performing significantly worse than NPMDs, who performed worse than HCs. On CVLT-II recall, PMD patients had poorer recall than NPMDs, who performed similar to HCs. This pattern held true for both free and cued recall, but no differences were apparent on the recognition trial. In contrast, PMDs and NPMDs both performed worse than HCs on story memory. PMDs performed similarly to NPMDs on immediate recall and recognition but worse on delayed recall. For executive function, PMDs demonstrated greater dysfunction than did both NPMD and HCs. Performance on measures of attention and working memory produced mixed results, with PMDs performing more poorly than HC and NPMDs on some tasks.

Cortisol

Significant relationships were observed between age and evening ($r = 0.253$, $P = 0.005$) and early morning cortisol ($r = 0.298$, $P = 0.001$). Thus, age was used as a covariate in cortisol analyses. As expected, PMD patients had a higher evening cortisol than did both HCs and NPMDs, who did not differ from one another (see Table 3). There were no significant differences between the three groups on early morning (0100–0900 hrs) cortisol. Correlations between cortisol and cognitive variables demonstrated moderate negative correlations but most did not achieve significance cutoff of $P < 0.005$ (see Supplementary Table 2). There was a significant correlation between mean evening and early morning cortisol, $r = 0.336$ ($P < 0.001$); however, the correlations were not so high as to exclude one from the regression model to predict cognition.

Genetics

Results of the Forward Linear Regression with GR and MR individually are displayed in Table 4 (*NR3C1*) and 5 (*NR3C2*). Of the *NR3C1* SNP's, rs10052957 and rs41423247 predicted performance on attention tasks, beyond the contributions of cortisol, age, gender, medication status or clinical measures. Other *NR3C1* SNPs sporadically predicted working memory (rs6198) and executive function (rs2918419). However, *NR3C1* SNPs did not contribute significantly to verbal memory. Interestingly, cortisol and gender were a strong predictors of verbal memory performance on CVLT list learning, whereas depression and gender were strong predictors of story memory.

In contrast, as seen in Table 5, *NR3C2* (MR) did not predict attention or working memory but did predict verbal memory. Variation in four separate SNPs (rs5525, rs4835488, rs 10213471 and rs17484245) predicted verbal memory performance for stories. When NR3C2 was added to the regression equation, gender was no longer a significant predictor of story memory. In addition, variation in rs7694064 was significant for predicting long-delay free recall in list learning measures, and the addition of this SNP usurped the variance that was related to gender.

Finally, FKBP5 appeared to not exert an effect on cognition. FKPB5 SNPs did not predict any measure of cognition.

As ethnicity has been known to influence genetic results, ethnicity was examined specifically among the participants with genetic data. In this subset, ethnicity did not differ across the three diagnostic ($\chi^2(6) = 5.88$, ns). Thus, separate analyses were run for the largest subset of patients, European Caucasians (% Caucasians: PMD = 72%, NPMD = 78%, HC = 67%). Results were generally similar between the full sample ($N = 80$) and the subsample with European Caucasians only ($N = 58$; see Supplementary Tables 3–5).

In the Caucasian sample, the overall prediction of cognition with NR3C1 SNPs held true for attention and working memory. Rs4142347 predicted attention for both the full and the Caucasian-only samples. However, additional SNPS achieved significance in the Caucasian-only sample, including rs33377, rs2918419 and rs12521436. Furthermore, consistent across the two samples, NR3C1 did not predict memory performance.

Similar results were found in the Caucasian-only and the overall samples when NR3C2 was put in the model to predict cognition (see Supplementary Table 4). Specifically, the five NR3C2 SNPs found to predict list memory and story memory were identical in the two samples. In the Caucasian-only sample, several additional SNPs emerged. In particular, rs227089 emerged as a relevant factor for list learning and long-delay list recall.

Finally, although FKPB5 did not emerge as a factor for any measure of cognition in the full sample, one FKBP5 SNP emerged as an important factor in the Caucasian-only analyses. Rs3800373 predicted delayed memory recall for list learning. Given the different distribution of rs3800373 among African- and Asian-American populations, it is likely that the reduced variability in the Caucasian-only sample led to the emergence of this SNP in predicting memory.

Haplotype analysis

For the NR3C1 haplotypes, there were no significant effects to potential haplotypes 1 or 5 and few significant effects for haplotype 4. NR3C1 haplotypes 4 and NR3C2 haplotype 2 significantly predicted performance on the Controlled Oral Word Association (FAS) test, accounting for 7.4% and 5.1% of the variance, respectively, after other variables were accounted for ($P \leqslant 0.001$; see Supplementary material). In addition, NR3C1 Haplotype 4 predicted 4.4% of the variance on the CVLT raw score trials 1–5 (learning over time) after depression level, early morning cortisol, and gender were controlled for ($P < 0.001$). No other significant findings emerged from haplotype analyses. Analyses were also performed for those who were confirmed as a carrier for a given Haplotype (that is, had two copies of the target allele on each SNP in the haplotype), and only the FAS test was significant for Haplotype 4. No other haplotype analyses were significant.

Table 4. Results of the Forward Regression Models predicting cognitive performance

Attention	*Full model*	*Beta value*	t-*value*	P-*value*	*% variance*
Digit Span Forward	F(2,72) = 6.60, $P < 0.001$, 26.8%				
	Mean evening cortisol	−0.332	−3.01	0.004	6.2
	Mean early morning cortisol	0.348	3.24	0.002	9.1
	rs10052957	−0.277	−2.73	0.008	6.8
	Rs41423247	0.228	2.18	0.033	4.8
TMT A	F(1,77) = 12.29, $P = 0.001$, 13.8%				
	Age	0.371	3.5	0.001	13.8
Working memory					
DS back	F(2,74) = 5.93, $P = 0.004$, 13.8%				
	Positive symptoms	−0.324	−2.97	0.004	8.5
	rs6198	−0.233	−2.14	0.036	5.3
LNS	F(2,77) = 16.49, $P < 0.001$, 30.0%				
	Positive symptoms	−0.390	−3.98	< 0.001	21.0
	Mean evening cortisol	−0.308	−3.15	0.002	9.0
Executive function					
Stroop Color Word	F(3,76) = 10.00, $P < 0.001$, 28.3%				
	Age	−0.292	−2.87	0.005	14.3
	Positive symptoms	−0.281	−2.82	0.006	10.3
	Evening cortisol	−0.207	−1.99	0.05	3.7
TMT B	F(2,75) = 5.73, $P = 0.005$, 13.3%				
	Evening cortisol	0.263	2.45	0.017	6.4
	Rs2918419	0.262	2.43	0.017	6.9
COWA FAS	F(1,78) = 10.24, $P = 0.002$, 11.6%				
	Mean evening cortisol	−0.341	−3.20	0.002	11.6
Verbal memory					
Total Trials 1–5	F(3,73) = 11.14, $P < 0.001$, 31.4%				
	HDRS Depression Score	−0.375	−3.87	< 0.001	15.3
	Mean evening cortisol	−0.311	−3.20	0.002	10.1
	Sex	−0.244	−2.52	0.014	6.0
SDFR	F(2,74) = 7.14, $P = 0.001$, 16.2%				
	Benzos	−0.266	−2.46	0.016	9.9
	Mean evening cortisol	−0.255	−2.34	0.021	6.3
SDCR	F(2,74) = 9.05, $P < 0.001$, 19.7%				
	Mean evening cortisol	−0.326	−3.11	0.003	12.3
	Sex	−0.273	−2.61	0.011	7.4
LDFR	F(3,73) = 7.21, $P < 0.001$, 22.9%				
	Benzos	−0.216	−2.00	0.049	10.8
	Early morning cortisol	−0.264	−2.52	0.014	6.5
	Sex	−0.245	−2.30	0.024	5.6
LDCR	F(2,74) = 9.43, $P < 0.001$, 20.3%				
	Mean evening cortisol	−0.358	−3.43	0.001	14.4
	Sex	−0.244	−2.34	0.022	5.9
Recognition	F(3,73) = 13.29, $P < 0.001$; 35.3%				
	rs17209258	−0.455	−4.77	< 0.001	15.0
	Mean evening cortisol	−0.401	−4.20	< 0.001	16.1
	Sex	−0.207	−2.20	0.031	4.3
LM					
Immediate memory	F(2,77) = 7.22, $P = 0.001$, 15.8%				
	HDRS Depression Score	−0.337	−3.22	0.002	10.8
	Sex	−0.224	−2.14	0.035	5.0
Delayed memory	F(2, 77) = 6.05, $P = 0.004$, 13.6%				
	HDRS Depression Score	−0.300	−2.83	0.006	8.5
	Sex	−0.225	−2.12	0.037	5.1
Recognition	F(2, 76) = 7.29, $P = 0.001$, 16.1%				
	Positive symptoms	−0.269	−2.48	0.015	10.7
	Mean evening cortisol	−0.239	−2.21	0.030	5.4

Abbreviations: COWA FAS, Controlled Oral Word Association Test using the letters F, A, S; CVLT, California Verbal Learning Test 2nd Edition; DS back, Digit Span Backwards from the Weschler Adult Intelligent Scale-III; HDRS, Hamilton Depression Rating Scale; GR, glucocorticoid receptor; LDCR, CVLT long-delay cued recall score; LDFR, CVLT long-delay free recall score; LM, Logical Memory Subtest of the Weschler Memory Scale-III; LNS, Letter Number Sequencing from the WAIS-III; SDCR, CVLT short-delay cued recall score; SDFR, CVLT short-delay free recall score; TMT A is the Trail Making Test–Subtest A; TMT B, Trail Making Test–Subtest B. Age, sex, depression severity, psychosis severity, mean evening cortisol, mean early morning cortisol, daily benzodiazepine medication use and *NR3C1* (GR) were all potential predictors. Statistics for the full model are given, and the predictors that made it into the model are immediately below. Beta and *t*- and *P*-values once the other predictors are accounted for are provided.

Table 5. Results of the Forward Regression Models for predicting cognitive performance

Attention	*Full model*	*Beta value*	t-*value*	P-*value*	*% variance*
Digit Span Forward	F(2,72) = 6.08, *P* = 0.004, 14.4%				
	Mean evening cortisol	−0.351	−3.04	0.003	6.3
	Mean early morning cortisol	0.0303	2.62	0.011	8.2
TMT A	F(1,75) = 11.60, *P* = 0.001,13.4%				
	Age	0.366	3.41	0.001	13.4
Working memory					
DS back	No significant model				
LNS	F(2,75) = 12.89, *P* < 0.001, 25.6%				
	Mean evening cortisol	−0.333	−3.26	0.002	16.0
	Positive symptoms	−0.316	−3.10	0.003	9.5
Executive function					
Stroop Color Word	F(3,74) = 8.54, *P* < 0.001, 25.7%				
	Age	−0.281	−2.68	0.009	13.5
	Positive symptoms	−0.231	−2.25	0.027	7.5
	Mean evening cortisol	−0.231	−2.16	0.034	4.7
TMT B	No significant model				
COWA FAS	F(2,75) = 7.04, *P* = 0.002, 15.8%				
	Mean evening cortisol	−0.333	−3.14	0.002	11.3
	Rs2070951	−0.211	−2.00	0.05	4.5
Verbal memory					
Total learning trials 1–5	F(3,71) = 10.83, *P* < 0.001, 31.4%				
	HDRS Depression Score	−0.376	−3.82	< 0.001	15.7
	Age	−0.309	−3.14	0.002	10.1
	Gender	−0.238	−2.42	0.018	5.7
SDFR	F(2,72) = 6.95, *P* = 0.002, 16.2%				
	Mean evening cortisol	−0.262	−2.28	0.020	9.8
	Benzos	−0.259	−2.35	0.022	6.4
SDCR	F(2,72) = 8.74, *P* < 0.001, 19.5%				
	Mean evening cortisol	−0.334	−3.15	0.002	12.9
	Sex	−0.258	−2.43	0.017	6.6
LDFR	F(3,71) = 7.99, *P* < 0.001, 25.2%				
	Benzos	−0.301	−2.86	0.006	10.4
	rs7694064	0.286	2.76	0.007	7.9
	Mean early morning cortisol	−0.270	−2.58	0.012	7.0
LDCR	F(3,71) = 7.79, *P* < 0.001, 24.8%				
	Mean evening cortisol	−0.319	−3.01	0.004	15.1
	Sex	−0.227	−2.19	0.032	5.1
	HDRS Depression Score	−0.218	−2.07	0.042	4.5
Recognition	F(1,73) = 9.14, *P* = 0.003, 11.1%				
	Mean evening cortisol	−0.334	−3.02	0.003	11.1
LM					
Immediate memory	F(2,75) = 11.66, *P* < 0.001, 23.7%				
	HDRS Depression Score	−0.373	−3.70	< 0.001	12.8
	rs4835488	0.331	3.28	0.002	10.9
Delayed memory	F(4,73) = 7.00, *P* < 0.001, 27.7%				
	HDRS Depression Score	−0.313	−3.06	0.003	11.2
	rs5525	−0.531	−3.44	0.001	5.2
	Rs10213471	0.398	2.59	0.012	6.4
	Mean evening cortisol	−0.227	−2.23	0.029	4.9
Recognition	F(2, 74) = 11.16, *P* < 0.001, 23.2%				
	Positive symptoms	−0.366	−3.59	0.001	14.6
	rs17484245	−0.2946	−2.88	0.005	8.6

Abbreviations: COWA FAS, Controlled Oral Word Association Test using the letters F, A, S; CVLT, California Verbal Learning Test 2nd Edition; DS back, Digit Span Backwards from the Weschler Adult Intelligent Scale-III; HDRS, Hamilton Depression Rating Scale; LDCR, CVLT long-delay cued recall score; LDFR, CVLT long-delay free recall score; LM, Logical Memory Subtest of the Weschler Memory Scale-III; LNS, Letter Number Sequencing from the WAIS-III; MR, mineralocorticoid receptor; SDCR, CVLT short-delay cued recall score; SDFR, CVLT short-delay free recall score; TMT A is the Trail Making Test–Subtest A; TMT B, Trail Making Test–Subtest B. Age, sex, depression severity, psychosis, mean evening cortisol, mean early morning cortisol, daily benzodiazepine use and *NR3C2* (MR) were all potential predictors. Statistics for the full model are given, and the predictors that made it into the model are immediately below. The Beta and *t*- and *P*-values are presented after accounting for the other predictors.

DISCUSSION

Data presented confirm, in a larger sample, previous reports of poorer cognitive performance and higher evening cortisol levels in PMD patients as compared with nonpsychotic depressed patients and HC. PMD patients performed significantly more poorly than did NPMD's and HC's on most measures of cognition, including working memory, executive function and verbal memory (list learning). Our data that NPMDs performed more

similarly to HC is contrary to some previous findings[36,37] but not all.[38] There is variability in the depression literature regarding what cognitive domains are impaired in depression, with severity[39,40] often being discussed as a main factor that mediates poor functioning. Our findings suggest that although depression level was an important variable in predicting total learning over the five trials on the CLVT-II and memory for stories, severity of depression accounted for little variance on most cognitive measures. Thus, severity of depression, which has been hypothesized to be a key factor in the variability of findings in the neuropsychological literature on depression, was not found to have a key role in a variety of cognitive measures in this study. This lack of association is consistent with several other studies.[41,42] Our study, however, included severely depressed patients (both nonpsychotic and psychotic) and as such we cannot comment on possible effect of depression severity across the spectrum.

Cortisol was a major contributor to cognition in the present study. PMD patients demonstrated higher evening cortisol levels than did both NPMD and HC's, who did not differ from each other. However, early morning cortisol did not differ between the groups. Regression analyses suggest that cortisol is potentially a stronger predictor of poor cognition than are clinical symptoms. These data point to a direct effect of cortisol on cognitive function that is independent of symptoms and medication usage. Cortisol accounted for between 5 and 16% of the variance of a variety of cognitive domains, and it generally accounted for higher levels of variance in memory tests. In the present data set, PMD was associated with both higher levels of depression and cortisol. Thus, the higher levels of depression and psychosis may serve as a proxy for higher levels of cortisol in depression and cognition studies.

Finally, genetic variation for NR3C1 (GR) and NR3C2 (MR) also at times contributed significantly to cognition, after accounting for the effects of cortisol. The degree of SNP contributions appears less than or equivalent to that of cortisol itself. In addition, N*R3C1* and *NR3C2* genetic variation did not predict cognition in the same manner. GR variation appeared more likely to predict attention and executive function tasks, suggesting involvement of the prefrontal cortex or cingulate; whereas MR variation was more likely to predict encoding and retrieval of longer term memories, functions more dependent on the hippocampus and medial temporal region. These results generally parallel GR and MR receptor distribution in primate brain[1] as well as previous reports on their putative roles in specific cognitive functions.[3]

Our results suggest roles for *NR3C1* polymorphisms in attentional tasks, including the Digit Span Forward, and tasks that require both attention and working memory, such as Digit Span Backward, consistent with Fortier *et al.*,[43] who reported that a specific haplotype of *NR3C1* SNP's (including rs6198) was associated with thought and attentional problems in ADHD. Furthermore, as indicated above, El Hage *et al.* [30] found significant interactions between *NR3C1* rs41423247 and COMT polymorphisms on working memory performance in HC.

In addition, one of our predictive GR SNP's (that is, rs10052957) on attentional tasks has been related to metabolic syndrome and metabolic risk factors[44] including higher fasting insulin concentrations and another SNP, rs2918419, contributed to executive function at the $P = 0.001$ level. The LD map for NR3C1 (Supplementary Figure 1) shows that rs10052957 and rs2918419 are in moderate but not complete LD. SNPs rs10052957 and rs2918419 are both intronic, suggesting that they are proxies for one or more functional genetic variants not assessed in our sample.

Cortisol elevation is well known to cause insulin resistance, and this may explain that metabolic syndrome and Type II diabetes (T2D) have been found to be more prevalent in patients with recurrent depression as compared with controls.[45] Depression may precede T2D,[46] independent of any effects of antidepressant medication.[47] T2D is an outcome of a metabolic syndrome; T2D, metabolic syndrome and depression are all associated with elevated cortisol levels.[48] In addition, elevated cortisol levels are associated with impaired cognition in HC, depressed patients and T2D.[49] T2D may also be associated with hippocampal damage.[50] A common genetic pathway underpinning all of these disorders has been hypothesized to stem from genetic variation in the HPA axis.[51]

NR3C1 SNP rs6198 in the 3'-untranslated region has perhaps been examined more widely than other *NR3C1* SNP's, and it has been implicated in risk of major depressive disorder,[52] smoking susceptibility,[53] binge eating[54] and attentional problems.[43] Each of these conditions has a high comorbidity with major depressive disorder, potentially related to shared genetic susceptibility.

Several *NR3C2* SNPs related specifically to verbal memory performance in the present study. Of these, only the rs10213471 promoter SNP might be expected to have a functional effect. The others are hypothesized to be proxies for as yet unknown genetic variants that are in LD. Of the SNP's studied herein, only the rs10212471 promoter SNP and the rs5525 synonymous exon SNP were in moderate in LD. Otherwise the significant NR3C2 SNPs represent largely independent effects (See LD map Supplementary Figure 2).

MR has been reported to have a major role in memory consolidation in rodents.[3,18] A number of years ago, we noted the relationships among insomnia, cortisol and memory impairment in geriatric subjects, suggesting MR involvement as it controls cortisol activity at the nighttime nadir.[55] Vogel *et al.*[56] linked *NR3C2* genetic variance in HC to negative memory bias, particularly in those with high life adversity. These findings are consistent with studies that find decreased MR expression in the hippocampus (and prefrontal cortex) in depressed patients.[22] Otte *et al.*[20] found that MR stimulation in man with fludrocortisone improved verbal memory and executive functioning compared with placebo. At last, Sun *et al.*[25] examined four large data sets on AD exploring other genes that can confer risk for AD beyond that of APOE, and concluded that one of the 10 potential risk genes was *NR3C2* (MR).

Beyond cortisol and genes predicting cognitive function, several other seemingly important factors were added to the model, with some influencing cognitive function. For example, gender was a consistent factor in predicting memory performance. In particular, when added to the model with NR3C1, gender predicted between 4.3 and 7.4% of the variance for memory for lists and stories. However, when in the model with NR3C2, much of the gender effect for story memory appears to be usurped by MR SNPs. Reasons for this finding need to be explored if such a finding is replicable. Medications is another factor that is theorized to have a role in cognition. In this particular case, we used benzodiazepine use because of its likelihood of having an effect on memory specifically. Indeed, benzodiazapine use accounted for about 10% of the variance in list memory, even when NR3C2 was is the model. Thus, medication use and gender factors, if replicable, warrant further understanding in relationship to cortisol and genetics on cognition. In addition, it is interesting to note differences in findings between the individual SNPs as compared with the haplotype analyses, despite using haplotypes previously observed in the literature.[33–35] It is possible that the reductions in sample size seen with using the haplotypes may have contributed to the limited observed significant findings, in part by the ambiguity that comes in inferring or estimating haplotypes.[57] Our results are consistent with others who have not found a relationship between haplotypes and clinical features of depression[58] nor with recurrence of major depression.[59] The conflicting results speak to the need for enhancement of genetic methods in psychiatric research, given the relatively small sample sizes in these populations.

The limited genetic research on cognition in psychiatric disorders, often point to interactions, particularly with environment, stress and other genes. This is particularly true for NR3C1 and

FKBP5. Although several other important models could be examined, including the interactions of *NR3C1* and *NR3C2* genes, FKBP5 and COMT genes, the available sample size with complete data (cognition, ratings, cortisol and genetics), precluded such important analyses, as did our lack of early childhood trauma data. Consequently, we decided to conduct more exploratory, forward regression analyses for each gene (GR and MR) separately. Further testing on larger, independent samples is needed to confirm the present findings and to extend upon them in understand the inherently complex interactions of genes that encode for specific receptors of the HPA axis as well as those that encode for receptors and enzymes of other neurotransmitter systems.

CONFLICT OF INTEREST

Although there is not a conflict of interest, there may appear to be one with Dr Schatzberg. He has a significant interest in Corcept Therapeutics, which licensed the use patent for mifepristone in psychotic major depression. Some of the data presented here were part of a larger study examining the effectiveness of mifepristone in psychotic major depression. No data on mifepristone's effectiveness are presented in the submitted report. Several authors have been named on a use patent related to the SNPs reported in this manuscript, including Alan Schatzberg, Greer Murphy and Jennifer Keller. Dr Greer Murphy has been a consultant for Brain Resource.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)