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The impact of mineralocorticoid receptor ISO/VAL genotype (rs5522) and stress on reward learning

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Research suggests that stress disrupts reinforcement learning and induces anhedonia. The mineralocorticoid receptor (MR) determines the sensitivity of the stress response, and the missense iso/val polymorphism (Ile180Val, rs5522) of the MR gene (*NR3C2*) has been associated with enhanced physiological stress responses, elevated depressive symptoms and reduced cortisol-induced MR gene expression. The goal of these studies was to evaluate whether rs5522 genotype and stress independently and interactively influence reward learning. In study 1, participants ($n = 174$) completed a probabilistic reward task under baseline (i.e. no-stress) conditions. In study 2, participants ($n = 53$) completed the task during a stress (threat-of-shock) and no-stress condition. Reward learning, i.e. the ability to modulate behavior as a function of reinforcement history, was the main variable of interest. In study 1, in which participants were evaluated under no-stress conditions, reward learning was enhanced in val carriers. In study 2, participants developed a weaker response bias toward a more frequently rewarded stimulus under the stress relative to no-stress condition. Critically, stress-induced reward learning deficits were largest in val carriers. Although preliminary and in need of replication due to small sample size, findings indicate that psychiatrically healthy individuals carrying the MR val allele, gene, which has been recently linked to depression, showed a reduced ability to modulate behavior as a function of reward when facing an acute, uncontrollable stressor. Future studies are warranted to evaluate whether rs5522 genotype interacts with naturalistic stressors to increase the risk of depression and whether stress-induced anhedonia might moderate such risk.

Keywords: Anhedonia, cortisol, depression, endophenotype, gene, glucocorticoid, HPA, mineralocorticoid, reward, stress

Several forms of psychopathology, including depression, are characterized by reward dysfunction (Diekhof *et al*. 2008). Reward processing is influenced by both genes (Forbes *et al*. 2009) and stress (Berenbaum & Connelly 1993), but how these factors interactively affect reward processing is largely unknown. Examining putative effects of gene × stress interactions on reward dysfunction might provide important clues about the etiology of depression (Hasler *et al*. 2004).

The mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) regulate the onset and termination, respectively, of the hypothalamic–pituitary–adrenal (HPA) axis stress response (Joëls *et al*. 2008). Antidepressant medications increase MR expression (DeRijk *et al*. 2008), which is associated with reduced depressive- and anxiety-like behavior as well as reduced corticosterone levels during stressful and basal conditions (Mitra *et al*. 2009; Rozeboom *et al*. 2007). Conversely, chronic stress results in reduced MR expression (Sterlemann *et al*. 2008) and MR antagonists increase basal and stress-induced cortisol levels and worsen antidepressant response (Arvat *et al*. 2001; Pace & Spencer 2005; Wellhoener *et al*. 2004).

The val allele of the iso/val missense single nucleotide polymorphism (SNP; Ile180Val, rs5522) within the MR gene (*NR3C2*) has been associated with (1) heightened endocrine and autonomic responses to acute stress (DeRijk *et al*. 2006), (2) diminished cortisol-induced MR gene expression (Arai *et al*. 2003; DeRijk *et al*. 2006) and (3) geriatric depressive symptoms (Kuningas *et al*. 2007). Based on these findings and the key role of MR in regulating stress responses, the main goal of the current studies was to examine whether MR iso/val genotype and an acute stressor (threat-of-shock) foster, individually and interactively, the emergence of an anhedonic phenotype. We developed two alternative hypotheses.

First, we hypothesized that val carriers would show elevated reward learning under no-stress conditions but blunted reward learning under stress. This hypothesis was derived from literature emphasizing an inverted U function between stress and performance, whereby too little or too much stress results in suboptimal function (Blascovich *et al*. 2003; Sapolsky 2003). Of primary relevance here, this inverted U function is believed to be driven by the balance of MR and GR occupancy (Sapolsky 2003). Specifically, saturated MR and low GR occupancy results in enhanced synaptic plasticity, potentiated ventral striatal neurotransmission and improved cognition, whereas high

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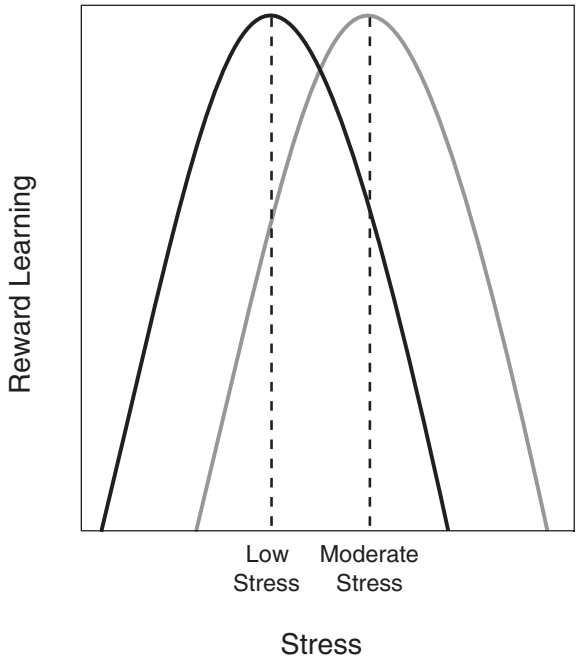

Figure 1: Graphical representation of the primary hypothesis for study 2. The black line represents val carriers; the gray line represents iso/iso homozygotes. Under low levels of stress, relative to iso/iso homogygotes, val carriers are expected to have heightened reward learning in light of higher MR saturation and low GR occupancy. With elevated stress, val carriers are hypothesized to be pushed away from the optimal point along the inverted U function resulting in reduced reward learning relative to iso homozygotes.

MR and GR occupancy is associated with reduced synaptic plasticity and cognitive deficits (Sapolsky 2003). Given the 10-fold greater affinity of cortisol to MRs, under low stress, MRs are saturated relative to GRs. The heightened stress reactivity and reduced cortisol-induced transactivation (Arai *et al*. 2003; DeRijk *et al*. 2006) characteristic of the val allele suggest that val carriers may have (1) elevated reward learning under no-stress conditions because of high MR saturation and low GR occupancy and (2) blunted reward learning under stress because of reduced cortisol-induced MR transactivation resulting in saturated MR and GR (Fig. 1). Alternatively, because the val allele has been associated with geriatric depressive symptoms (Kuningas *et al*. 2007), we hypothesized that val carriers might show overall blunted reward learning irrespective of stress manipulation.

Materials and methods

Participants

Study 1

The final sample consisted of 174 participants (Table 1) recruited from Harvard University and the surrounding community. Participants were excluded if they presented: current medical illness, attention-deficit hyperactivity disorder (ADHD), head injury, loss of consciousness, seizures, current alcohol/substance abuse or dependence, smoking, use of psychotropic medications during the last 2 weeks, pregnancy or left handedness (Chapman & Chapman 1987). Subjects received $5.00 or course credit for participation and 'won' money (average $6.00) during the reward task. A prior paper focusing on event-related potential (ERP) data collected on a subsample of these subjects ($n = 47$) has appeared (Santesso *et al*. 2008).

Study 2

The final sample consisted of 53 healthy female participants (Table 2). All participants were right-handed (Chapman & Chapman 1987) and reported to be of European ancestry (i.e. two parents of European ancestry) and to be free of color blindness, past or present neurological, psychiatric, hormonal or metabolic disturbances. Only females were recruited because differences in HPA axis system function as well as behavioral responses to stress are theorized to contribute to the twofold rate of depression found in women relative to men (Nolen-Hoeksema *et al*. 1999; Young & Korszun 2010). Only Caucasians were recruited to limit potential confounds of population stratification (Freedman *et al*. 2004). (We note that this procedure does not fully exclude the potential for population stratification, which could be addressed through a genomic control analysis; Reich

Table 1: Study 1 demographic and self-report data

	Iso/Val	Iso/Iso	Statistics	*P* value
N	44	130		
Ethnicity (% Caucasian)	68%	71%	$\chi^2(1) = 0.04$	0.84
Age	22.70 ± 5.10	22.56 ± 5.35	$t(171) = 0.15$	0.88
Gender (% female)	57%	63%	$\chi^2(1) = 0.54$	0.46
BDI-II	9.00 ± 8.98	7.98 ± 7.05	$t(170) = 0.77$	0.45
MASQ GDA	18.93 ± 6.92	18.96 ± 6.21	$t(171) = -0.03$	0.98
MASQ AA	23.32 ± 8.63	22.78 ± 6.36	$t(171) = 0.44$	0.66
MASQ GDD	24.55 ± 10.65	22.74 ± 9.30	$t(171) = 1.07$	0.29
MASQ AD	55.95 ± 15.54	55.69 ± 14.20	$t(171) = 0.10$	0.92
PSS	23.42 ± 9.19	23.00 ± 7.99	$t(170) = 0.29$	0.77

Numbers represent means and SD. The ethnicity distribution across the entire sample was: Caucasian = 70.1% African American = 11.5%; Asian = 11.5%, Native American = 1.1%, Hispanic = 2.9% and multiracial = 2.9%.
BDI-II, beck depression inventory-II total score. MASQ, mood and anxiety symptom questionnaire.
AA, anxious arousal; AD, anhedonic depression; GDA, general distress anxiety; GDD, general distress depression; PSS, perceived stress scale.

Table 2: Study 2 demographic and self-report data

	Iso/Val	Iso/Iso	Statistics	*P* value
N	8	45		
Ethnicity (% Caucasian)	100	100		
Gender (% female)	100	100		
Age	22.50 ± 1.51 (22, 20–25)	22.04 ± 1.76 (22, 19–25)	$t(51) = 0.69$	0.50
Education	15.69 ± 0.80 (16, 14–16.5)	15.62 ± 1.55 (16, 10–19)	$t(51) = 0.12$	0.91
BDI	1.50 ± 1.77 (1, 0–5)	3.02 ± 4.32 (1, 0–25)	$t(50) = -0.98$	0.33
MASQ GDA	14.63 ± 2.26 (14.5, 12–18)	15.64 ± 3.85 (15, 11–27)	$t(50) = -0.72$	0.48
MASQ AA	18.63 ± 2.00 (18.5, 17–23)	18.70 ± 2.14 (18, 17–26)	$t(50) = -0.10$	0.92
MASQ GDD	15.25 ± 2.05 (15, 13–19)	17.11 ± 4.47 (16, 12–34)	$t(50) = -1.15$	0.26
MASQ AD	60.25 ± 9.89 (61, 41–74)	55.32 ± 8.32 (57, 35–72)	$t(50) = 1.50$	0.14
PSS	16.63 ± 3.58 (17, 9–22)	18.07 ± 6.07 (17.5, 4–33)	$t(51) = -0.65$	0.51

Education = years of education completed. See Table 1 for explanation of additional abbreviations. Numbers represent means and SDs. Values in parentheses represent the median and range.

& Goldstein 2001). Participants completed two sessions and were paid $10 per hour for their time and 'won' $15 during the reward task. A report on this sample focusing on variation across the *CRHR1* gene and ERP data collected in this study is in preparation (Bogdan *et al*. 2008). Across both studies, participants provided written informed consent to procedures approved by the Committee on the Use of Human Subjects in Research at Harvard University.

Procedure

Study 1

Participants were given instructions and told that the objective of the probabilistic reward task was to win as much money as possible. Following the task, participants completed several questionnaires, including the Perceived Stress Scale (PSS; Cohen *et al*. 1983), Beck Depression Inventory II (BDI-II; Beck *et al*. 1996), and Mood and Anxiety Symptom Questionnaire (MASQ; Watson *et al*. 1995). At the end of the session, subjects provided a saliva sample for genetic analyses and a Structured Clinical Interview for the Diagnostic and Statistical Manual, Fourth Edition (DSM-IV) (SCID; First *et al*. 2002) was administered to ensure no past or present axis I disorder.

Study 2

The study consisted of two sessions. In session 1, the SCID (First *et al*. 2002) was administered to ensure no past or present axis I disorder (participants with past minor alcohol abuse, i.e. one symptom meeting threshold more than 2 years ago, were included, $n = 2$). Eligible participants then completed questionnaires including the MASQ and PSS and provided a saliva sample for DNA analysis.

In session 2 (on average, 5.25 days after session 1; SD: 4.04), participants performed the probabilistic reward task under a stress (threat-of-shock) and no-stress condition (counterbalanced across subjects). In the stress condition, two electrodes were attached to the back of participants' right hand, 0.5 cm apart. Before the stress condition, shock intensity was adjusted individually; specifically, shocks were administered to participants starting at 0.4 milliamperes and increased in intensity up to 4.0 milliamperes or until a participant defined it as '*highly aversive or unpleasant, but not painful*'. Participants were instructed that they would receive one to three electrical shocks during the stress condition and that the intensity of shocks would increase over time. Additionally, because stressors that are uncontrollable are associated with an enhanced stress response and anhedonic behavior in non-human animals (Anisman & Matheson 2005) and humans (Breier *et al*. 1987; Dickerson & Kemeny 2004), participants were told that shocks were randomly triggered by the computer and thus unrelated to their performance. For each participant, one shock was administered at the end of block 1. In an effort to maintain stress throughout the experiment, following block 2, the experimenter informed the participant: '*I am aware you did not receive a shock during the last block of the task. As a result, it is highly probable that you will receive a shock during the next block*'. Before the no-stress condition, participants were informed that it was impossible to receive any shocks. Moreover, if the no-stress condition occurred first, the shock device was never introduced into the room; if the no-stress condition was second, the shock device was removed from the room at least 15 min before the no-stress condition.

Subjects completed the state forms of the Spielberger Trait Anxiety Inventory (STAI; Spielberger *et al*. 1970) and Positive and Negative Affect Scale (PANAS; Watson *et al*. 1988) four times: immediately before (pre-task) and after (post-task) the stress and no-stress conditions. Post-task questionnaires were modified to ask participants about their mood during the task. Participants were given a 15-min break following completion of the first task to ensure that mood returned to baseline levels. If a participant's mood had not returned to baseline, an additional 5 min were provided before the second behavioral task began. Lastly, participants completed the BDI-II (Beck *et al*. 1996). Skin conductance (SC) levels were recorded throughout both task conditions from the distal phalanges of the left index and middle fingers.

Probabilistic reward task

A probabilistic reward task rooted in signal detection theory was used to measure reward learning, i.e. an individual's propensity to modulate behavior according to prior reinforcement history (Pizzagalli *et al*. 2005; Tripp & Alsop 1999). In addition to standard measures of hit rate and reaction time (RT), this task allows for the computation of *discriminability*, which indexes the ability to perceptually distinguish two stimuli, and *response bias*, which reflects the participant's tendency to select one stimulus regardless

of actual stimulus presentation. Importantly, unequal frequency of reward following correct identifications of two stimuli produces a systematic preference (response bias) for the response paired more frequently with reward (Macmillan & Creelman 2005; Pizzagalli *et al*. 2005). An asymmetric reward schedule was used to induce a response bias (Pizzagalli *et al*. 2005); specifically in each block, correct identification of one stimulus (the 'rich' stimulus) was rewarded three times more frequently than the other stimulus (the 'lean' stimulus). Response bias, our main variable of interest, was used to objectively assess the modulation of behavior as a function of prior reinforcement history.

Reward processing can be parsed into distinct neurochemical, neuroanatomical and psychological components, such as wanting (anticipation), liking (consumption) and the learning of stimulus–reward relationships (Berridge & Kringelbach 2008). The present task provides an empirical measure of reward learning. Of relevance to the current study, in community, clinical, and student samples reward learning has been found to be: (1) blunted in participants with depression (Pizzagalli *et al*. 2009); (2) blunted under an acute laboratory stressor (Bogdan & Pizzagalli 2006; Morris & Rottenberg 2009); (3) heritable and genetically associated with perceived stress (Bogdan & Pizzagalli 2009), and influenced by the interaction of corticotropin-releasing hormone type 1 receptor (CRHR1) genotype and stress (Bogdan *et al*. 2008).

Study 1

The probabilistic reward task was identical to that described in an independent sample (Pizzagalli *et al*. 2005). The task consisted of 300 trials divided into three 100-trial blocks. Each trial began with the presentation of a fixation cross in the middle of the screen for 500 milliseconds. A mouthless cartoon face then appeared for 500 milliseconds before a short (11.00 mm) or long (13.00 mm) mouth was presented for 100 milliseconds. The mouthless face remained on the screen until a response was made. Reward feedback was displayed for 1500 milliseconds on rewarded trials followed by a blank screen for 250 milliseconds; on non-rewarded trials, a blank screen was displayed for 1750 milliseconds. According to the reinforcement schedule, in each block, correct identification of either the short or long stimulus was rewarded (*Correct!! You won 5 cents*) three times more frequently (i.e. 30 vs. 10) than the other stimulus (counterbalanced across subjects).

Study 2

The task was similar to that used in study 1 with the following exceptions. First, because participants completed the task during both a stress and a no-stress condition, two different stimuli (mouth and nose) were used as targets (long mouth: 11.00 mm, short mouth: 10.00 mm; long nose: 5.31 mm, short nose: 5.00 mm) to avoid carryover effects. Second, to minimize participants' fatigue, each block consisted of 80 trials for a total of 240. Four additional trials (trials 81–84) were appended to the first block in each condition (excluded from analyses). During trial 81 of the stress condition, all participants received a 1-second shock. For each block, the asymmetrical reinforcement schedule consisted of 24 rewards for the rich stimulus and 8 rewards for the lean stimulus. Third, participants were rewarded with 7.5 cents. Finally, the fixation cross was presented for 750–900 milliseconds, and the mouthless (or noseless) face following stimulus presentation was left on the screen for 1500 milliseconds.

Apparatus

The task was presented on an IBM 2.4-GHz PC using E-PRIME software (version 1.2; Psychology Software Tools, Inc., Pittsburgh, PA, USA). Responses were made with a response pad (PST Serial Response Box; Psychology Software Tools, Inc.). Shock was delivered via a finger stimulator (Coulbourn Instruments, E13-22, Whitehall, PA, USA) and pre-gelled electrodes (Kendall Foam 4103; Tyco Healthcare Group LP, Mansfield, MA, USA). PSYLAB hardware (SAM SC5) and software (PSYLAB8) were used for the collection, measurement and analysis of SC data (Contact Precision Instruments, Boston, MA, USA). Saliva samples for DNA analyses were collected with Oragene collection kits (OG-250 and OG-100; DNA Genotek, Ottawa, Ontario, Canada).

Data collection and reduction

Behavioral data

A two-step procedure was used to identify outlier responses: (1) trials with RTs shorter than 150 milliseconds or longer than 1500 milliseconds were excluded and (2) for each participant, remaining data were logarithmically transformed and trials with RTs exceeding mean ± 3 SD were excluded. *Response bias* and *discriminability* were computed as follow (Hautus 1995; Pizzagalli *et al*. 2005):

Response bias:

$$\log b = \frac{1}{2}\log\left(\frac{(\text{Rich}_{\text{correct}}+0.5)\times(\text{Lean}_{\text{incorrect}}+0.5)}{(\text{Rich}_{\text{incorrect}}+0.5)\times(\text{Lean}_{\text{correct}}+0.5)}\right)$$

Discriminability:

$$\log d = \frac{1}{2}\log\left(\frac{(\text{Rich}_{\text{correct}}+0.5)\times(\text{Lean}_{\text{correct}}+0.5)}{(\text{Rich}_{\text{incorrect}}+0.5)\times(\text{Lean}_{\text{incorrect}}+0.5)}\right)$$

Skin conductance

Data were recorded at 300 Hz and subsequently resampled at 10 Hz. SC responses were identified with an automated procedure within PSYLAB software using three data points (onset, slope and peak). This automated process avoids the accidental detection of an SC response because of noise or movement. The frequency of nonspecific SC responses/minute was computed for each block within each condition.

Genotyping

DNA obtained from saliva samples was purified, extracted and hydrated; when not in use, it was stored at −80°C. MR rs5522 primers (F: ACGTTGGATGCTCATGACACATGATAGGGC; R: ACGTTGGATGTTATGTCTGACTCTGGGAGC) were designed using SPECTRODESIGNER software (Sequenom, San Diego, CA, USA). Following a polymerase chain reaction, an iPLEX massEXTEND reaction was performed (extension primer: CATGATAGGGCTTTTAACAA). After baseline correction and peak identification, Sequenom SPECTROTYPER software was used to analyze resulting spectra. Genotyping was undertaken in conjunction with several other studies on related topics. rs5522 was the only MR SNP that was typed because of *a priori* hypotheses (DeRijk *et al*. 2006; Kuningas *et al*. 2007).

Statistics

Chi-square tests or *t* tests were performed to evaluate possible group differences on demographics and self-report measures. For the probabilistic reward task in study 1, an analysis of variance (ANOVA) with *Genotype* and *Block* (1, 2 and 3) was conducted. For study 2, an analogous ANOVA with the additional within-subject factor of *Condition* (stress and no-stress) was run. To assess the effectiveness of the stress manipulation in study 2, *Genotype* (iso/iso, val carrier) × *Condition* (stress, no-stress) × *Time* (pre-task, post-task) ANOVAs were conducted separately on the STAI and PANAS scores, whereas a *Genotype* × *Condition* × *Block* (1, 2 and 3) ANOVA was conducted on nonspecific SC response frequency. Significant ANOVA effects were followed-up with *t* tests.

Results

Study 1

Genotype groups did not differ on self-report measures (Table 1). Replicating prior studies in independent samples (Pizzagalli *et al*. 2005), participants successfully modulated their behavior according to reinforcement contingencies;

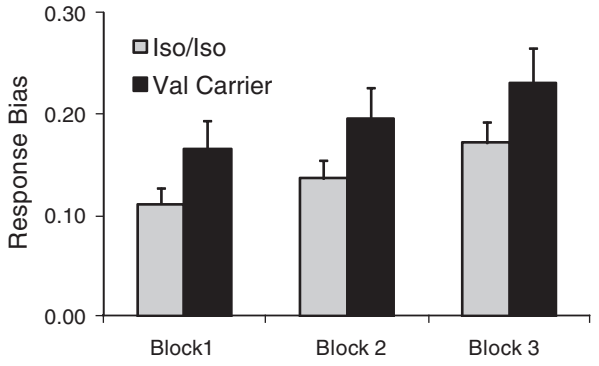

Figure 2: Effect of MR genotype on response bias in study 1. Black bars represent val carriers ($n = 44$); gray bars represent iso homozygotes ($n = 130$). Error bars represent SEs of the mean.

for response bias, a main effect of *Block* emerged ($F_{2,344} = 4.16$, $P = .018$) driven by higher response bias in blocks 3 (mean ± SD: 0.19 ± 0.23; $t(173) = 3.08$, $P = 0.002$] and 2 [0.15 ± 0.21; $t(173) = 1.80$, $P = 0.073$] compared with block 1 (0.12 ± 0.19). Critically, a main effect of *Genotype* was also found ($F_{1,172} = 4.77$, $P = 0.030$, Cohen's $d = 0.38$) because of higher response bias in val carriers (0.19 ± 0.15) relative to iso homozygotes (0.14 ± 0.15) (Fig. 2). The main effect of *Genotype* was confirmed when considering Caucasian subjects only ($F_{1,118} = 4.47$, $P < 0.04$, Cohen's $d = 0.45$), suggesting that findings were not confounded by population stratification. For discriminability, only a trend emerged for *Block* ($F_{2,344} = 2.86$, $P = 0.064$), because of higher discriminability in block 3 (0.85 ± 0.28) and 2 (0.85 ± 0.30) relative to block 1 (0.81 ± 0.29), both Ps < 0.06.

Study 2

Demographic, self-report, and stress data

Genotype groups did not differ on any demographic or self-report variables (Table 2). An independent t test showed that group differences in shock intensity approached significance [$t(48) = 1.97$, $P = 0.055$; Cohen's $d = 0.77$]; relative to iso homozygotes (2.31 ± 0.90 milliamperes), val carriers selected a lower level of shock intensity to be highly aversive (1.66 ± 0.47 milliamperes). The ANOVA on STAI scores produced main effects of *Condition* ($F_{1,51} = 18.17$, $P < 0.001$) and *Time* ($F_{1,51} = 38.28$, $P < 0.001$). These main effects were qualified by a *Condition* × *Time* interaction ($F_{1,51} = 12.90$, $P = 0.001$; Fig. 3a). *Post hoc* tests revealed that, as intended, participants reported significantly higher STAI scores during the stress (44.67 ± 9.70) relative to no-stress (39.39 ± 7.16) condition [$t(52) = 4.44$, $P < 0.001$], whereas no significant differences emerged for the pre-stress assessments [no-stress: 34.51 ± 7.11, stress: 35.46 ± 6.96; $t(52) = 1.22$, $P = 0.23$]. Additionally, for both the stress and no-stress condition, STAI scores significantly increased over the course of the experiment, both ts > 5.66, both Ps < 0.001. A *Time* × *Condition* × *Genotype* trend emerged ($F_{1,51} = 3.89$, $P = 0.054$), but follow-up t tests showed no significant differences between genotypes, all Ps > 0.26.

For PANAS NA, main effects of *Condition* ($F_{1,51} = 25.13$, $P < 0.001$) and *Time* ($F_{1,51} = 17.42$, $P < 0.001$) emerged. A significant *Condition* × *Time* interaction qualified these main effects ($F_{1,51} = 17.11$, $P < 0.001$; Fig. 3b). Participants reported elevated NA scores during the stress (14.63 ± 4.06) relative to the no-stress (12.09 ± 2.39) condition as well as elevated NA scores before the stress (11.81 ± 1.77) relative to the no-stress (11.30 ± 1.41) condition, both ts > 2.25, both Ps < 0.03. Additionally, for both the stress and no-stress condition, NA scores significantly increased over the course of the experiment, both ts > 2.75, both Ps < 0.01. In addition, trends for a *Time* × *Condition* × *Genotype* ($F_{1,51} = 3.65$, $P = 0.062$) and *Time* × *Genotype* ($F_{1,51} = 2.95$, $P = 0.092$) interaction emerged. Independent samples t tests showed, however, no differences between genotype groups, all Ps > 0.15. For PANAS positive affect, no significant effects emerged, all Ps > 0.21.

When considering nonspecific SC responses, the expected main effect of *Condition* emerged ($F_{1,42} = 4.47$, $P = 0.04$), due to significantly more responses in the stress (4.01 ± 2.58) compared with no-stress (3.32 ± 2.18) condition (Fig. 3c). A trending effect for *Genotype* was observed ($F_{1,42} = 3.39$, $P = 0.07$, Cohen's $d = 0.72$); as expected based on prior findings (DeRijk *et al*. 2006), val carriers (4.56 ± 3.76) had more SC responses than iso homozygotes (2.77 ± 1.91). Collectively, these findings indicate that the stress manipulation was successful; participants reported elevated anxiety and negative affect and had more nonspecific SC responses in the stress relative to no-stress condition. With the exception of a trend for higher SC responses in val carriers, the effects of the stress manipulation were similar across genotypes.

Probabilistic reward task

Replicating prior findings (Bogdan & Pizzagalli 2006), there was a significant main effect of *Condition* for response bias ($F_{1,51} = 6.32$, $P = 0.015$), due to reduced response bias toward the more frequently rewarded stimulus in the stress (0.02 ± 0.16) relative to no-stress (0.14 ± 0.16) condition (reported in more detail in Bogdan *et al*., in preparation). Most importantly, this effect was qualified by a *Genotype* × *Condition* interaction ($F_{1,51} = 5.12$, $P = 0.028$; Fig. 4). Relative to iso homozygotes, val carriers had significantly lower response bias in the stress condition [−0.05 ± 0.16 vs. 0.08 ± 0.15; $t(51) = 2.27$, $P = 0.03$, Cohen's $d = 0.89$]. No differences emerged within the no-stress [val carrier: 0.18 ± 0.22 vs. iso homozygote: 0.10 ± 0.15; $t(51) = 1.41$, $P = 0.16$, Cohen's $d = 0.55$] condition. Within-group analyses indicated that val carriers tend to have lower response bias in the stress relative to no-stress condition [$t(7) = 2.29$, $P = 0.056$, Cohen's $d = 1.22$], whereas iso homozygotes did not differ between conditions [$t(44) = 0.33$, $P = .74$]. Figure 4c displays the distribution of response bias in the stress and no-stress condition for iso homozygotes and val carriers; the correlation between stress and no-stress response bias was not significant in either group (iso homozygotes: $r = -0.27$, $P = 0.07$; val carriers: $r = -0.10$, $P = 0.82$).

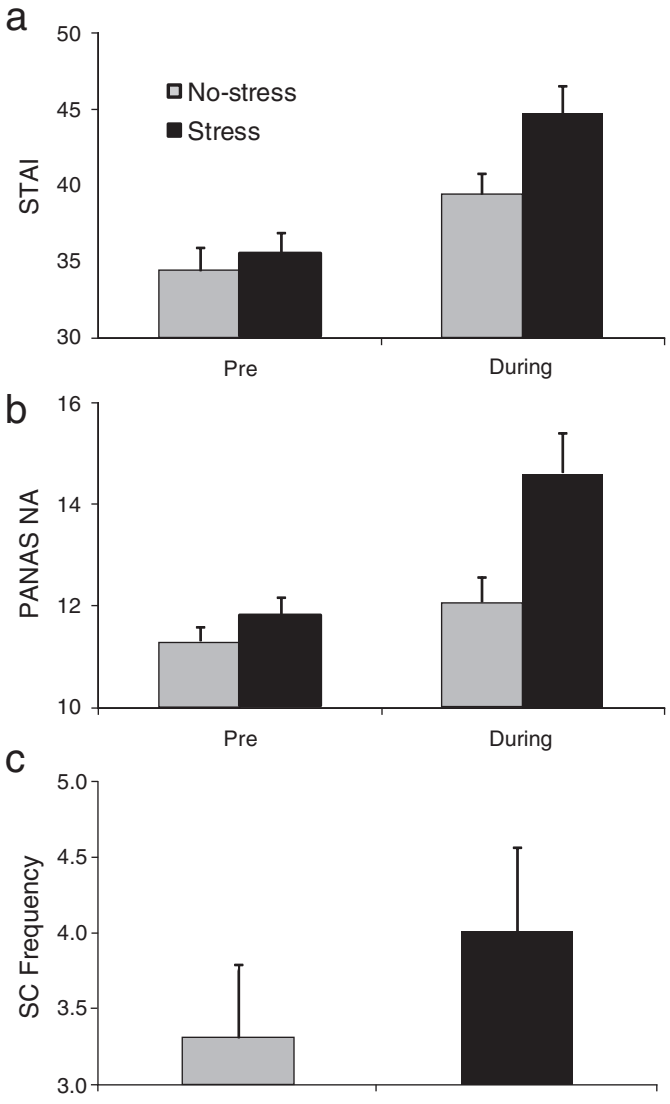

Figure 3: Study 2 stress manipulation data ($n = 53$). (a) Significant *Condition* × *Time* interaction on state anxiety, as measured by the STAI. (b) Significant *Condition* × *Time* interaction on state negative affect, as measured by the PANAS. (c) Significant main effect of *Condition* on nonspecific SC response frequency. In all panels, black bars represent the stress condition and gray bars represent the no-stress condition. Error bars represent SEs of the mean.

Control analyses

For discriminability, a main effect of *Block* emerged ($F_{2,102} = 3.80$, $P = 0.026$), because of elevated discriminability in blocks 3 (0.41 ± 0.02) and 2 (0.40 ± 0.02) relative to block 1 (0.35 ± 0.02), both Ps < 0.02. Additionally, a *Condition* × *Block* × *Genotype* interaction emerged ($F_{2,102} = 3.76$, $P = 0.027$). Independent *t* tests showed, however, no differences between genotype groups in any block.

Finally, three sets of hierarchical regressions were conducted to confirm that MR genotype differences in response bias were not confounded by differences in discriminability, shock intensity, SC responses or other genotypes. In all regressions, possible nuisance variables were entered in the first step, MR genotype was entered in the second step and the difference in response bias between stress and no-stress was entered as the criterion variable. In the first model, discriminability in both conditions was entered in the first step. In the second, shock intensity and SC responses were entered in the first step. Finally, in the third model, three CRHR1 SNPs (i.e. rs12938031, rs10445364 and rs4076452) recently associated with stress-induced reward learning deficits (Bogdan *et al*. 2008) were entered in the first step. Findings confirmed that the val allele still explained unique variance of stress-induced response bias reductions after accounting for (1) discriminability ($\Delta R^2 = 0.09$, $\Delta F_{1,49} = 5.08$, $P = 0.03$), (2) shock intensity and SC responses in both conditions ($\Delta R^2 = 0.23$, $\Delta F_{1,41} = 12.45$, $P = 0.001$) and (3) CRHR1 SNPs ($\Delta R^2 = 0.05$, $\Delta F_{1,44} = 3.24$, $P = 0.08$).

Discussion

The main goal of the present study was to assess how an acute laboratory stressor (threat-of-shock) and MR

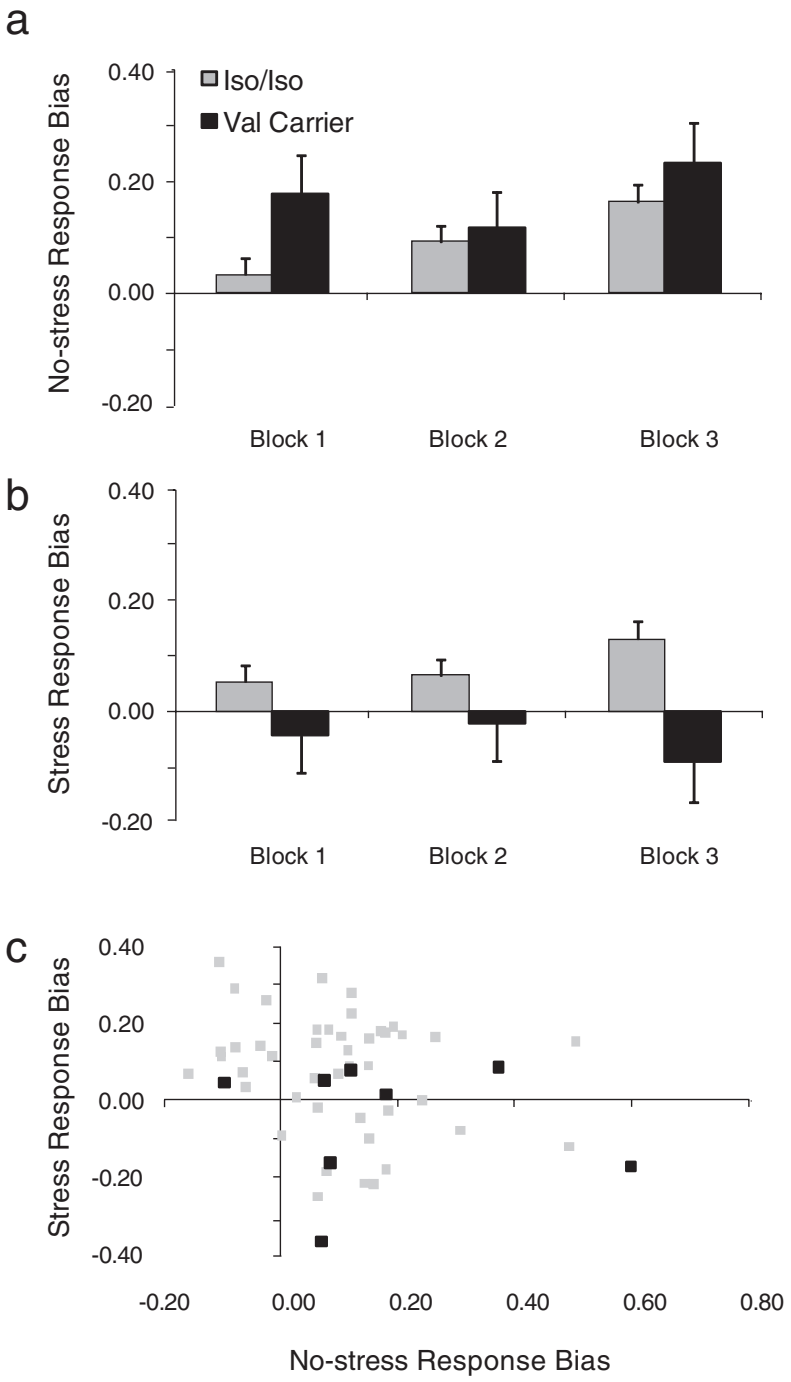

Figure 4: Effects of MR and stress manipulation on response bias in study 2. (a) No-stress condition. (b) Stress condition. (c) Scatterplot displaying the distribution of stress (*y*-axis) and no-stress (*x*-axis) response bias scores. In all panels, black bars/squares represent val carriers ($n = 8$); gray bars/squares represent iso homozygotes ($n = 45$). Error bars represent SEs of the mean.

genotype independently and interactively affect reward learning. Three main findings emerged. First, replicating prior findings (Bogdan & Pizzagalli 2006), psychiatrically healthy participants developed a weaker response bias toward a more frequently rewarded stimulus when performing the task under acute stress (reported and discussed in more detail in Bogdan *et al*., in preparation), which elicited the intended emotional and physiological responses. Second, in both studies, under no-stress conditions, val carriers had enhanced reward learning relative to iso homozygotes, although groups significantly differed only in study 1. Third, a *Genotype* × *Condition* interaction emerged in study 2: relative to iso homozygotes, val carriers showed significantly lower reward learning under the stress but not no-stress condition; moreover, although iso homozygotes performed similarly under the two conditions, val carriers showed lower

response bias in the stress relative to no-stress condition. Importantly, analyses controlling for CRHR1 SNPs recently associated with stress-induced anhedonia (Bogdan *et al*., in preparation) suggest that MR iso/val genotype contributed to the present findings above and beyond the effects of CRHR1 SNPs.

Consistent with a wealth of animal literature indicating that stress induces anhedonic behavior (Anisman & Matheson 2005), emerging human research suggests that stressors can negatively affect components of reward processing and activation in mesolimbic dopaminergic pathways implicated in reinforcement learning, incentive motivation and hedonic responses (Berenbaum & Connelly 1993; Bogdan & Pizzagalli 2006; Dillon *et al*. 2009; Mehta *et al*. 2009; Pizzagalli *et al*. 2007; Pruessner *et al*. 2004). Interestingly, recent preclinical data show that stress hormones influence dopamine function as well as approach-related behavior (Peciña *et al*. 2006; Wanat *et al*. 2008) raising the possibility that genetic variation in regulators of the HPA axis may affect reward processing.

The findings emerging from the current studies identify the MR iso/val polymorphism (rs5522) as an important moderator of reward processing and stress-induced reward learning deficits. Consistent with our primary hypothesis, MR val carriers showed elevated reward learning under basal conditions but were susceptible to stress-induced deficits in reward learning. Critically, these findings emerged in spite of unaffected abilities to perceptually distinguish the two stimuli, as evidenced by the lack of group differences in discriminability (see also, the hierarchical regression analyses accounting for differences in discriminability), suggesting that response bias findings were not confounded by stress-induced changes in perceptual or attentional processes. Rather, val carriers were less able to modulate their behavior as a function of the asymmetric reinforcement schedule, i.e. were less responsive to rewards under stress.

Our primary hypothesis was inspired by prior work suggesting an inverted U pattern linking stress and performance (Blascovich *et al*. 2003), which might be driven by an imbalance of MR and GR occupancy (Sapolsky 2003). According to these mechanisms, under basal conditions, val carriers might show heightened reward learning because of high MR occupancy and low GR occupancy leading to enhanced performance. However, when challenged by even a small amount of stress, val carriers might be pushed away from the optimal point along the inverted U function (Fig. 1) and show relatively higher GR occupancy relative to iso homozygotes, leading to reduced reward learning under stress. Prior findings highlighting reduced cortisol-induced gene expression of MR and enhanced endocrine and autonomic responses to psychosocial stress in val carriers (DeRijk *et al*. 2006) are consistent with this interpretation.

The alternative hypothesis that val carriers would show blunted reward learning irrespective of condition was not supported. It has been suggested that elevated geriatric depressive symptoms in val carriers may be the result of lifelong exposure to elevated cortisol (Kuningas *et al*. 2007). This interpretation is consonant with sensitization ('kindling') processes, whereby repeated stressors and depressive episodes lead to changes leaving individuals susceptible or 'kindled' to develop later depressive episodes independent of stress (Kendler *et al*. 2000; Post 1992). The lack of reward deficits under basal conditions in young adult val carriers is not entirely surprising. It is possible that stress is required to elicit reward processing deficits in young val carriers, whereas the lifelong accumulated effects of an exaggerated stress response may leave geriatric val carriers susceptible to depressive symptoms (Kuningas *et al*. 2007). Regardless of the interpretation, the current data suggest that MR val carriers are more prone to display anhedonic behavior when exposed to an uncontrollable acute stressor. Whether such deficits might increase their risk of developing depression is currently unknown and warrants further study.

This report has several limitations. First, neither study evaluated endocrine stress responses; given that MR iso/val genotype moderates endocrine responses to stress (DeRijk *et al*. 2006), it will be critical to test whether heightened endocrine activity is a key mediating factor leading to reduced reward learning. This study was able to address this issue more distally by evaluating SC levels; critically, hierarchical regression analyses indicated that genotype differences in SC levels did not account for reduced reward learning.

Second, study 2 is limited by a relatively small sample and limited generalizability given its sole composition of healthy Caucasian females. Thus, the findings from study 2 are susceptible to type I error and require replication. The small sample size might also have contributed to the finding that, although val carriers show elevated reward learning under no-stress conditions in both studies, the effect was significant only in study 1 despite a moderate effect size in study 2 (Cohen's $d = 0.55$). The inclusion of psychiatrically healthy participants implies that any interpretation of stress-induced anhedonia with respect to the etiology of depression is entirely speculative. However, the use of a healthy sample provides the advantage of evaluating possible vulnerability factors without the confounding effects of past or current depression, thus eliminating the possibility that any group differences might be the consequence of the disorder. Because most of the participants tested here had not passed the greatest vulnerability period for depression, it is unclear whether the current val carriers might be at increased the risk of depression or rather represent a 'resilient' group. Longitudinal studies will be required to answer this critical question.

Third, in light of the importance of MR/GR occupancy ratio for stress responses and coping (de Kloet *et al*. 2007; Sapolsky 2003; Wang *et al*. 2008; Young *et al*. 2003), research with larger samples will be needed to test possible interactions between MR and GR polymorphisms. Finally, and more importantly, the generalizability and clinical relevance of the current findings await investigations in depressed samples evaluated for naturalistic stressors with contextual measures of life stress (e.g. the Life Events and Difficulties Schedule; Brown & Harris 1978). Despite these limitations, this report is the first, to our knowledge, to test the effects of the MR iso/val polymorphism and stress on the ability to modulate behavior as a function of reward. Whether MR iso/val genotype might increase the risk of depression through the emergence of stress-induced anhedonia is a testable hypothesis that warrants further investigation.

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