Systems/Circuits

Valence-Specific Effects of *BDNF* Val^{66}Met Polymorphism on Dopaminergic Stress and Reward Processing in Humans

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Brain-derived neurotrophic factor (BDNF) levels in dopaminergic (DA) cells within the ventral tegmental area (VTA)/nucleus accumbens (NAc) circuitry appear to be a candidate mechanism for the neuroadaptive changes that follow stress and reward responses in animal models. However, the role of the *BDNF* gene variants in responses to salient cues through DA neurotransmission in humans remains unexplored. Here, we studied the effect of the common functional *BDNF* Val^{66}Met (rs6265) polymorphism on rewarding experiences in the striatum and DA-mediated responses to stress. Seventy-two healthy controls were genotyped for the *BDNF* Val^{66}Met polymorphism and underwent the monetary incentive delay task during an functional magnetic resonance imaging (fMRI) session. Forty-nine of them also underwent a sustained pain challenge with and without placebo administration with potential analgesic properties during PET measures of DA $D_{2/3}$-receptor-mediated neurotransmission. Neuroimaging results revealed a significant effect of *BDNF* (Met^{66} carriers > Val/Val) on brain responses during the anticipation of monetary losses, baseline $D_{2/3}$ receptor availability, and pain-stress-induced DA release in the NAc. Conversely, BDNF Met^{66} carriers showed no activation in response to monetary gains and a blunted DA response to the analgesic placebo in the NAc. These results provide initial human evidence regarding the effect of the *BDNF* Val^{66}Met polymorphism on DA-mediated responses to stress, its cognitive regulation by positive expectations, and the anticipatory responses to monetary gains and losses in the VTA-NAc pathway. Our results are of relevance to the neurobiology of stress and reward interactions and the pathophysiology of stress-related disorders.

Key words: **BDNF Val66Met; dopamine; nucleus accumbens; pain; reward; stress**

Introduction

Brain-derived neurotrophic factor (BDNF) plays a key role in synaptic plasticity and the survival and function of dopaminergic (DA) neurons within the ventral tegmental area–nucleus accumbens (VTA-NAc) pathway. In animal models, striatal *in vivo* infusions of BDNF locally augment spontaneous electrical activity of midbrain DA neurons (Shen et al., 1994), enhance DA turnover (Altar et al., 1992), and elevate DA-activity-dependent release (Goggi et al., 2002). BDNF levels in DA cells in the VTA-NAc pathway also seem to be involved in the neuroadaptive changes occurring after intermittent brief episodes of social defeat stress, persistent chronic subordination (Berton et al., 2006; Krishnan et al., 2007; Miczek et al., 2011), and responses to rewarding stimuli (Horger et al., 1999; Pierce and Bari, 2001; Lu et al., 2004). BDNF injections into the VTA or the NAc enhance cocaine-induced locomotion (Pierce and Bari, 2001) and responses to cocaine cues for up to 30 d after the cessation of cocaine use (Lu et al., 2004). In addition, BDNF heterozygote knock-out mice are also less responsive to cocaine's rewarding effects (Horger et al., 1999; Hall et al., 2003).

In humans, mesolimbic activity and DA neurotransmission in the NAc are robustly engaged during the anticipation of a number of salient cues regardless of valence (Knutson et al., 2000; Scott et al., 2006; Scott et al., 2007b; McCabe et al., 2009; Spreckelmeyer et al., 2009). However, a potential role of BDNF on the processing of salient cues of different valences as it relates to DA neurotransmission has not been studied in humans. A common single-nucleotide polymorphism (SNP) in the *BDNF* human gene, Val^{66}Met (rs6265), codes a substitution from valine (Val) to methionine (Met) at codon 66. It is postulated that Met substitution leads to inefficient trafficking of BDNF to secretory granules and reduced activity-dependent BDNF release (Egan et al., 2003; Chen et al., 2004; Chen et al., 2006). This functional

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polymorphism has been associated with interindividual variations in cognitive function (Egan et al., 2003), stress reactivity (Duman and Monteggia, 2006; Frielingsdorf et al., 2010; Colzato et al., 2011), and reward processing (Gasic et al., 2009).

Here, we studied the effect of the *BDNF* Val[66]Met functional polymorphism on anticipatory responses to monetary gains and losses during the fMRI monetary incentive delayed (MID) task and striatal DA neurotransmission during two challenges of opposite valence known to activate DA release, a stress-pain challenge (Scott et al., 2006), and placebo administration with expectations of analgesia (Scott et al., 2008) using PET and the DA $D_{2/3}$-selective receptor radiotracer [^{11}C]-raclopride. Based on the animal literature and assuming effects regardless of valence, we initially hypothesized that, compared with *Val* homozygotes, decreased BDNF-dependent activity in *Met* carriers would result in lower functional responses during the anticipation of monetary gains and losses, lower baseline DA tone with a compensatory upregulation of $D_{2/3}$ receptors at baseline, and potentially lower DA release during both a painful stressor and placebo administration. The effects of *BDNF* Val[66]Met were expected within the VTA DA projections to the NAc, medial prefrontal cortex (mPFC), hippocampus (Hipp), and amygdala (AMY; Russo and Nestler, 2013) during the MID and in the NAc in the DA PET studies.

Materials and Methods

Subjects

Seventy-two subjects (34 females), age 20–40 years (mean ± SE, 26 ± 0.5) were genotyped for *BDNF* Val[66]Met SNP (rs6265) and completed the MID task. Pain reports were collected (see "Experimental Design") in a subsample of 49 subjects (28 females, age 26.45 ± 0.7 years) that participated in two PET scanning sessions during a pain-stress challenge with and without placebo administration as described previously (Scott et al., 2008). Results on 20 of the 49 subjects in the present sample were part of a previous study examining the effects of placebo administration on μ-opioid and DA neurotransmission (Scott et al., 2008). Main effects of placebo in the present sample replicated those in the previous study (Scott et al., 2008) and therefore are not reported here. BDNF Val[66]Met gene effects were not studied in this sample (Scott et al., 2008) due to insufficient power. In addition to completing physical and neurological examinations, study participants underwent screening using the nonpatient version of the Structured Clinical Interview for *DSM-IV*. Participants had no history of or current medical, neurological, or psychiatric illnesses, including substance abuse or dependence and had an alcohol intake of <5 drinks per week. Participants also had no first-degree family history of psychiatric illness. Women had regular menstrual cycles of 26–32 d duration and had not used hormonal birth control for at least 1 year. We attempted to study women during the follicular phase using menstrual cycle diaries at screening and confirmatory progesterone levels at each PET scanning session (progesterone levels of ≤1 ng/ml were confirmed in 23/28 women). Protocols were approved by the University of Michigan Investigational Review Board and the Radioactive Drug Research Committee and written informed consent was obtained from all subjects.

Genotyping

BDNF Val[66]Met SNP (rs6265) was genotyped in all subjects using the Illumina Golden Gate Assay platform, the Addictions Array content of 130 genes (1350 SNPs), and 186 Ancestry Informative Markers (AIMs), which have been described previously (Hodgkinson et al., 2008). Genotyping accuracy was confirmed by replicate genotyping of 10% of the total sample with a completion rate of >93% (mean 99.4%, median 100%) and replicates showed no errors at this loci. Because the sample was predominantly Caucasian (75%) and most of the non-Caucasians were African Americans (18%), the European and African ethnic factors (European and African AIM scores) were included as a continuous covariate in statistical analyses to account for the variability in allele frequencies across ethnicities.

MID and fMRI data acquisition and analysis

MID. Seventy-two participants completed a version of the MID (Knutson et al., 2000). Each subject completed 2 runs consisting of 72 trials lasting 6 s each. A trial consisted of a cue representing a monetary value (small: ±$0.20, medium: ±$1.00, and large: ±$5.00, null: $0), followed by an anticipation phase and a neutral target requiring button press with their right thumb. Subjects were then informed of their success on the preceding trial, in which they either gained or avoided losing the cued amount of money for monetary gain or loss trials, respectively. In the null trials, subjects experienced no monetary gain or loss but were still instructed to respond to the target. Subjects successfully hit the target an average of 45 ± 24% of the gain trials and 44 ± 24% of the loss response trials. Their average reaction time on gain trials was (165 ± 41 ms) and on loss trials was (165 ± 43 ms).

fMRI data acquisition. The blood oxygenation level-dependent (BOLD) signal was measured using a Signa 3-tesla scanner (General Electric) with standard RF coil using a T2* weighted pulse sequence (single-shot combined spiral in/out, gradient echo; repetition time = 2 s; echo time = 30 ms; flip-angle = 90 deg; field-of-view = 20 cm; 64-by-64 image matrix; slice thickness = 4 mm; 29 oblique-axial slices; Glover and Law, 2001). This imaging protocol was selected to minimize signal loss due to magnetic susceptibility effects (Noll, 2002). Field map images were collected to correct for B0 inhomogeneities (Noll et al., 1991).

Data were reconstructed offline, slice-time corrected to the middle slice (Aguirre and D'Esposito, 1999), and realigned to the first volume of each run to correct for intrascan movement using Statistical Parametric Mapping (SPM)-based algorithms (Friston et al., 1995). Each session was visually inspected for artifacts and screened for excessive head movement (mean translational or rotational instantaneous head movement >0.55 mm). High resolution anatomical MRI studies were also acquired using an axial spoiled gradient recall T1-weighted sequence (echo time, 3.4 ms; repetition time, 10.5 ms; inversion time, 200 ms; flip angle, 25°; number of excitations, 1; using 124 contiguous images, 1.5 mm thickness).

To allow comparisons between individuals, a subject's MRI and functional images were coregistered and anatomically normalized by warping the anatomical T1-weighted image to a standard stereotactic space (Montreal Neurological Institute, MNI) using SPM8 (Wellcome Department of Cognitive Neurology, University College, London). Finally, functional images were smoothed with a Gaussian kernel (full width at half maximum 6 mm) to reduce residual interindividual variability. Smoothed functional images were band-pass filtered with a 128 s high-pass filter to eliminate low-frequency signals.

Pain and placebo experimental design

Forty-nine subjects underwent two PET scans with [^{11}C]-raclopride. The experimental design, in the absence or presence of placebo, consisted of a painful condition starting at minute 45 and maintained for 20 min after radiotracer administration by a computer-controlled delivery system through the infusion of medication-grade hypertonic saline solution (5%) into the left masseter muscle. In this model of sustained deep somatic pain, the intensity of the painful stimulus is standardized across subjects (Zhang et al., 1993; Stohler and Kowalski, 1999). Briefly, volunteers are asked to rate their pain intensity every 15 s from 0 (no pain) to 100 (most intense pain imaginable) using an electronic 0–100 visual analog scale (VAS) placed in front of the scanner gantry during the pain condition. Initially, the subject-specific settings of the closed-loop system for maintaining muscle pain were established. This consisted of measuring each subject's response to a standard 0.15 ml bolus of 5% sodium chloride injected over a 15 s period as an impulsive input while recording the subject's pain intensity response every 15 s. A suitable infusion rate for the maintenance of pain over time was then estimated by comparing the subject's response to the mean response of 65 subjects of the same age range exposed to the same bolus. From that point on, the adaptive controller depended on feedback from subjects. The subject ratings of pain intensity every 15 s were fed back to the computer via an analog-digital board, which then changed the infusion rate to maintain pain at similar

levels over time. The same individual infusion profiles generated during the pain challenges were used for the studies with placebo administration (Scott et al., 2008).

During the placebo condition, subjects were given the following instructions before administration of the placebo: "We are studying the effect of a pain relief medication. This medication is thought to have analgesic effects through the activation of natural brain systems that suppress pain." The placebo condition consisted of the introduction of 1 ml of 0.9% isotonic saline into 1 of the intravenous ports every 4 min, with the volunteer being made aware that this was the case, starting 2 min before the pain challenges and lasting for 15 s each time. Subjects were aware that the study drug was to be administered because they were alerted by a computer-generated human voice recording, followed by a second-by-second count of the infusion timing (15 s). Based on our prior experience using a fully randomized design (Zubieta et al., 2005), the placebo administration followed the pain challenge without placebo; this provided the subjects with a frame of reference for the expectation of analgesic effects. Each subject underwent four pain challenges, two of them with placebo administration, as described previously (Scott et al., 2008), but only the results of two of the sets are reported here, those associated with [^{11}C]-raclopride PET scanning. The order of each pair of pain and pain + placebo studies was randomized. Pain and pain + placebo studies were separated by 2 h (changes in nondisplaceable binding potential [BP_{ND}] do not persist in subsequent scans after the pain stressor; Scott et al., 2007a).

Immediately after the pain and the pain + placebo challenges, subjects completed the McGill Pain Questionnaire (MPQ; Melzack and Torgerson, 1971). This measure, together with a 0–100 VAS pain intensity rating acquired every 15 s in the absence and presence of placebo, was used as the primary end point for the assessment of placebo responses. Levels of expectancy and effectiveness of placebo were rated before and after the placebo administration, respectively, with the following questions: for expectancy, "From 0 to 100 how effective do you think the treatment will be?"; for effectiveness, "From 0 to 100 how effective was the treatment?"

PET data acquisition and preprocessing

The two 90 min PET studies per subject were acquired (HR^+ scanner; Siemens) in 3D mode (reconstructed full-width/half-maximum resolution, ~5.5 mm in plane and 5.0 mm axially), with the septa retracted and scatter correction. Participants were positioned in the PET scanner gantry and two intravenous (antecubital) lines were placed. A light forehead restraint was used to eliminate intrascan head movement. [^{11}C]-raclopride was synthesized at high specific activity by the reaction of *O*-desmethyl raclopride with [^{11}C]-methyl triflate. Then, 15.0 ± 2.2 mCi was administered in each of the imaging procedures, with a mass of raclopride of 0.20 ± 0.15 μg/kg per image. These levels ensured that the compounds were administered in tracer quantities; that is, subpharmacological doses occupying <1% of the available receptors. Fifty percent of the [^{11}C]-raclopride dose was administered as a bolus, with the remainder delivered as a continuous infusion by a computer-controlled automated pump to more rapidly achieve steady-state tracer levels. For each study, 21 sets of dynamic scans were acquired with an increasing duration (4 30 s frames, 3 1 min frames, 2 2.5 min frames, 8 5 min frames, and 4 10 min frames). Images were reconstructed using iterative algorithms (brain mode; Fourier rebinning algorithm with ordered-subsets expectation maximization, four iterations, and 16 subsets; no smoothing) into a 128 × 128 pixel matrix in a 28.8-cm-diameter field of view. Attenuation correction was performed through a 6 min transmission scan (Ge^{68} source) obtained before the PET study and with iterative reconstruction of the blank/transmission data, followed by segmentation of the attenuation image. Small head motions during PET were corrected by an automated computer algorithm for each subject before analysis and the images were coregistered with the same software (Minoshima et al., 1993). Time points were then decay corrected during reconstruction of the PET data. Image data were transformed on a voxel-by-voxel basis into two sets of parametric maps, a tracer transport measure (K_1 ratio) and a receptor-related measure (BP_{ND}), the latter using data obtained from 35–45 min (baseline) or 45–90 min (pain stress ± placebo) after tracer administration. This measure was obtained using the ratio of brain activity to activity in the cerebellum minus 1 (Carson et al., 1997; Watabe et al., 2000). Using the bolus-continuous infusion protocol described in the experimental design, the slope of the Logan plot becomes linear ~5–7 min after tracer administration and is proportional to the receptor concentration divided by its affinity for the radiotracer as follows: $[(f_2B_{max}/K_d) + 1]$, where f_2B_{max}/K_d is the BP_{ND} (Innis et al., 2007) or receptor availability *in vivo*, B_{max} is the receptor concentration, and K_d is the receptor-ligand dissociation constant. The term f_2 refers to the concentration of free radiotracer in the extracellular fluid and is considered to represent a constant and very small value.

The K_1 and BP_{ND} images for each experimental period and the MRIs acquired on the 3 tesla scanner described in fMRI data acquisition, above, were coregistered to each other and to the International Consortium for Brain Mapping stereotactic atlas orientation (Meyer et al., 1997).

Three receptor-related measures were calculated: (1) DA $D_{2/3}$ BP_{ND} during the pain challenge (45–90 min., scan 1); (2) DA $D_{2/3}$ BP_{ND} during the pain + placebo challenge (45–90 min., scan 2); and (3) baseline DA $D_{2/3}$ BP_{ND} at equilibrium (35–45 min., scan 1). DA release during pain and placebo administration was assessed by calculating the difference between baseline (control) and pain conditions and between pain and pain + placebo conditions. Reductions in the *in vivo* availability of DA receptors after an acute challenge are thought to reflect DA release and competition between radiotracer and endogenous ligand for the receptor sites (Narendran and Martinez, 2008). A mask that included only regions with specific DA $D_{2/3}$-receptor-binding potential ($BP_{ND} > 0.2$) was used.

Statistical analyses

For the fMRI data, statistical analysis proceeded in two stages. At the first level, a general linear model was used to generate individual subjects' activation maps. The anticipation phase for each of the different monetary values was modeled separately as regressors of interest and was convolved with the hemodynamic response function. Six regressors modeling residual effects of head motion were included as nuisance parameters. The contrasts of interest generated with the model were: (1) anticipation of large gain versus neutral and (2) anticipation of large loss versus neutral. At the second level, contrast images were place into MNI space using the transformation matrix derived from the linear and nonlinear warping transformation matrices and random-effects analysis was used to determine group effects, resulting in statistical parametric (*t* or *F*) maps. Statistical test were also applied to the two primary contrasts of interest, large gain minus neutral and large loss minus neutral conditions. A mask excluded the cerebellum and brainstem below the midbrain because these regions were not well represented. A voxel-by-voxel *t* test (fMRI) and mixed model of variance (PET) analysis were performed using SPM8 (Wellcome Department of Cognitive Neurology, University College, London) and MATLAB software (The MathWorks). To account for differences in sample sizes, we chose the unequal variance option in SPM for statistical analysis. Sex and European and African AIM factors were included as covariates. No global normalization was applied to the data.

The effects of *BDNF* Val[66]Met were hypothesized to take place within the VTA dopaminergic projections to the NAc, mPFC, Hipp, and AMY (Russo and Nestler, 2013) during the MID and within the NAc in the DA PET studies (because of the selective binding of [^{11}C]-raclopride in the striatum). The summary statistical maps in these regions were thresholded at $p < 0.001$ uncorrected for multiple comparisons (Friston, 1997), with a voxel extent >10 voxels; for other regions, $p < 0.05$ false discovery rate (FDR)/FWE correlation was considered significant. These data were extracted for quantification of regional changes in BOLD activation and BP_{ND}, plotting, examination of potential outliers, and further statistical analyses using SPSS version 20.0 statistical software. ANCOVA models were performed on the psychophysical and extracted imaging data. Data are shown as the mean ± 1 SD. Genotype was included as the between-subject factors and sex and European and African AIMs were included as the covariates of no interest. Statistical significance was considered at $p < 0.05$.

We used the SPSS 20.0 macro *Mediate.sbs* (http://afhayes.com/spss-sas-and-mplus-macros-and-code.html) to estimate the path coefficients and the size of the indirect effect of the mediator model X (*BDNF* Val[66]Met) on *Y* (MPQ scores immediately after the pain challenge) through *Z* (*BDNF* Val[66]Met effect on NAc DA release during pain).

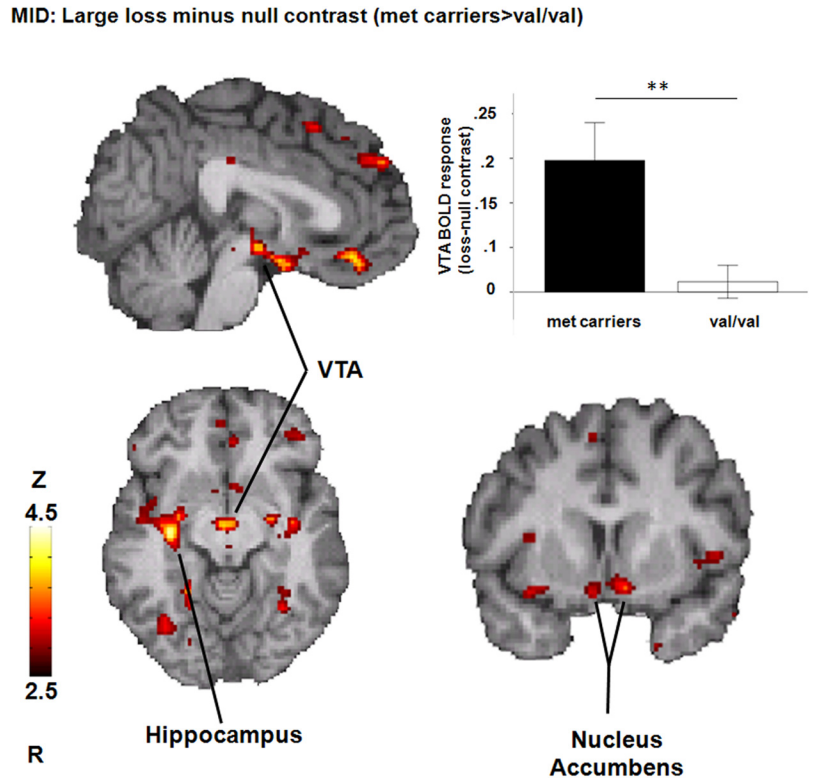

Figure 1. *BDNF* Val[66]Met effects during the anticipation of large monetary loss minus null condition (for all regions, $p < 0.001$; $n = 72$). The bar graph shows regional BOLD activation in the VTA for the large monetary loss minus null condition for each genotype (**$p < 0.005$).

Table 1. Effects of *BDNF* Val[66]Met during the anticipation of losses, stress, and placebo-induced DA release and $D_{2/3}$ receptor availability

	Cluster size[a]	*Z*-score[b]	Coordinates[c]
Anticipation of losses (Met[66] > Val/Val)			
mPFC	464	3.55	−2, 36, −14
VTA	216	3.46	2, −12, −10
NAc/hypothalamic region	104	3.42	−8, 8, −14
			−2, 0, −16
Hipp	744	3.9	34, −16, −10
Parahippocampus	152	3.55	30, 4, −14
Pain-induced DA release (Met[66] > Val/Val)			
NAc	38	3.58	−8, 6, −3
	25	3.23	13, 11, −4
Placebo-induced DA release (Val/Val > Met[66])			
NAc	237	4.19	14, 7, −1
	56	3.75	−12, 6, −5
D2/D3 receptor availability (Met[66] > Val/Val)			
NAc	402	3.46	−19, 8, −13

[a]Cluster size in cubic millimeters.
[b]Two-sided voxel-level *Z*-score at peak voxel (for all regions, $p < 0.001$, uncorrected).
[c]MNI coordinates of peak voxel.

Results

Genotyping

Seventy-two healthy volunteers were genotyped for the *BDNF* Val[66]Met polymorphism (18 were Met[66] carriers and 54 Val/Val homozygotes) and completed the MID fMRI task. Forty-nine of these volunteers were scanned with PET and completed pain reports; 11 subjects carried at least one Met allele and 38 were homozygotes for the Val allele. The *BDNF* Val[66]Met genotype distribution was in Hardy-Weinberg equilibrium in the two samples ($n = 72$, $\chi^2 = 0.1$, $p = 0.7$; $n = 49$: $\chi^2 = 2$; $p = 0.16$) and there were no significant differences between the two genotype groups with respect to sex, age, or European and African AIM scores.

Main effect of the MID and Val[66]Met (rs6265) on anticipation of gains and losses during the MID

Consistent with previous reports (Knutson et al., 2000), the anticipation of large monetary gains in the whole-brain analysis was associated with activation in the following regions (for all regions, $p < 0.05$, FDR corrected): the NAc bilaterally (with an area of activation that extended to the medial thalamus): MNI peak coordinates 8, −16, 10; cluster size 12.160 mm^3; *Z*-score 5.1; the anterior cingulate cortex: MNI peak coordinates 0, −4, 50; cluster size 8.696 mm^3; *Z*-score = 5; the occipital cortex: MNI peak coordinates 32, −94, 8; cluster size 23.008 mm^3; *Z*-score = 5.8; and the primary motor area: MNI peak coordinates −48, −8, 50; cluster size 3.256 mm^3; *Z*-score = 4.6. Anticipation of large monetary losses also induced regional activation in (for all regions, $p < 0.05$, FDR corrected): the NAc bilaterally (extending to the medial thalamus): MNI peak coordinates −10, 10, 0; cluster size 25.840 mm^3; *Z*-score = 6.3; in the anterior cingulate cortex: MNI peak coordinates 0, 10, 44; cluster size 3.872 mm^3; *Z*-score = 4.2; in the AMY: MNI peak coordinates 30, 0, −14; cluster size 440 mm^3; *Z*-score = 4.4; in the occipital cortex: MNI peak coordinates −4, −86, −10; cluster size 17.632 mm^3; *Z*-score = 5.5; and in the primary motor area: MNI peak coordinates −46, −2, 50; cluster size 3.208 mm^3; *Z*-score = 4.1.

During the anticipation of monetary loss (large loss minus null condition), a whole-brain analysis showed a significant effect of *BDNF* Val[66]Met (Met[66] varriers > Val/Val; for all regions, $p < 0.001$; Fig. 1, Table 1) in the mPFC, the VTA, the NAc and its extention to the hypothalamic region, the Hipp, and the parahippocampus. No significant effects were found for the opposite contrast (Val/Val > Met[66]) or during anticipation of reward (large gain minus null condition).

Val[66]Met (rs6265) effects on $D_{2/3}$ receptor availability at baseline and DA release during pain stress and placebo analgesia

A voxel-by-voxel analysis within the striatum during the control condition revealed a significant effect of *BDNF* Val[66]Met ($n = 49$, Met[66] carriers > Val/Val) on baseline $D_{2/3}$ receptor BP_{ND} in the left ventral striatum (MNI peak coordinates −19, 8, −13; cluster size 402 mm^3; *Z*-score = 3.46) and right ventral striatum, although at a lower threshold (Fig. 2, Table 1). Baseline $D_{2/3}$ receptor BP_{ND} in the ventral striatum was not

associated with pain ratings or changes in pain ratings during placebo administration with expectation of analgesia.

During the pain challenge, a voxel-by-voxel analysis within the striatum showed a significant effect of *BDNF* Val[66]Met ($n = 49$, Met[66] carriers > Val/Val) on pain-induced $D_{2/3}$ system activation maps in the NAc bilaterally [left NAc: MNI peak coordinates −8, 6, −3; cluster size 38 mm^3; Z-score = 3.58; in the right NAc: MNI peak coordinates 13, 11, −4; cluster size 25 mm^3; Z-score = 3.23 (Fig. 3, Table 1)]. Results remained significant after controlling for baseline DA BP_{ND} in the NAc.

The magnitude of DA release in the NAc by the pain stressor was positively correlated with MPQ scores during the pain condition in the left NAc (MPQ total: $r = 0.41$; $p = 0.003$; MPQ sensory: $r = 0.39$; $p = 0.006$; MPQ pain effect: $r = 0.30$; $p = 0.04$; Fig. 3). We then conducted a mediation analysis to test whether DA release in the NAc mediates the effect of *BDNF* Val[66]Met on the subjective pain experience, measured by the MPQ. The mediation analysis confirmed the *BDNF* Val[66]Met effect on DA release in the NAc during the sustained pain challenge (coefficient = −0.18, $t = -2.72$, $p = 0.009$) and the relationship between stress-induced DA release in the NAc and MPQ ratings (coefficient = 23.8, $t = 3.2$, $p = 0.002$). The mean indirect effect (coefficient = 0.13) from the bootstrap analysis was significant, with a 95% confidence interval excluding zero (−1.13 to −8.12) and the direct effect (coefficient = 0.3.5) of *BDNF* Val[66]Met on MPQ scores was not significant (p = 0.0.33). This suggests an indirect-only mediation (Zhao et al., 2010), a form of mediation that is consistent with full mediation in Baron and Kenny's procedure (Baron and Kenny, 1986). These results therefore show that the effect of *BDNF* Val[66]Met on pain reports is mediated by DA release in the NAc during pain.

During placebo administration, the imaging data analysis showed a significant effect of *BDNF* Val[66]Met, in this case, Val/Val> Met[66] carriers ($n = 49$), on placebo-induced $D_{2/3}$ system activation maps in the NAc bilaterally, where Met[66] carriers showed an overall deactivation of the DA system during placebo administration compared with Val carriers ($n = 49$, Fig. 3, Table 1). Results remained significant after controlling for baseline DA BP_{ND} in the NAc. Placebo-induced changes in DA neurotransmission in the NAc were not associated with changes in the subjective experience of pain during placebo administration.

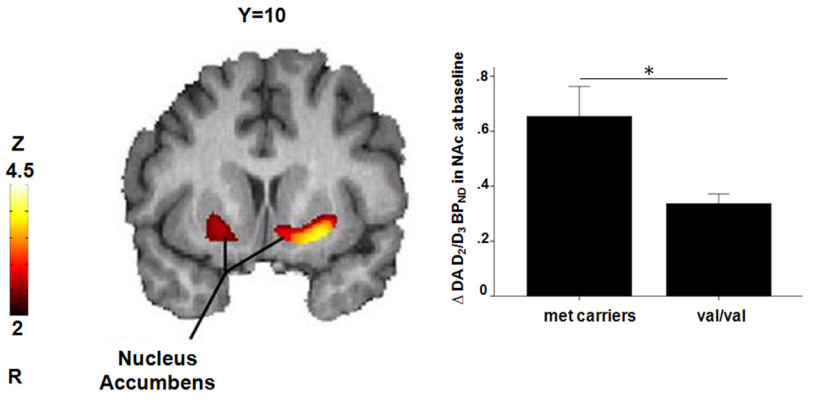

Figure 2. Effects of *BDNF* Val[66]Met (Met[66] carriers > Val/Val) on DA $D_{2/3}$ receptor BP_{ND} in the bilateral ventral striatum ($n = 49$). Data are expressed as means ± SEM. Significant differences between genotype groups are marked with asterisks (*$p < 0.05$).

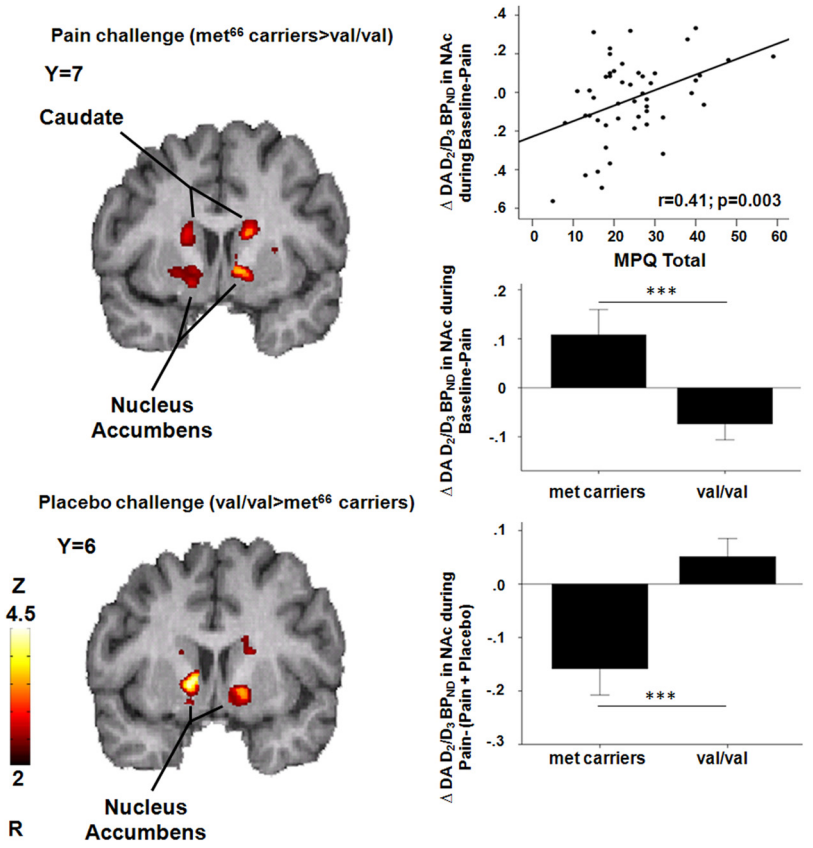

Figure 3. Effects of *BDNF* Val[66]Met on DA release. Top, Effects of *BDNF* Val[66]Met on stress-induced DA release in the caudate bilaterally and the NAc ($n = 49$). DA release in the left NAc was positively correlated with the MPQ total score. The bar graph shows *BDNF* Val[66]Met effects (Met[66] carriers > Val/Val) on DA activation during the pain challenge (means ± SEM). Significant differences between genotype groups are marked with asterisks (***$p < 0.001$). Bottom, Effects of *BDNF* Val[66]Met on DA release during placebo administration during and experimental pain in the NAc bilaterally ($n = 49$). Reductions in the average momentary pain intensity ratings acquired every 15 s were negatively correlated with placebo-induced DA release in the right NAc in Met[66] carriers ($r = -0.65$, $p = 0.03$), but not in Val homozygotes ($r = -0.025$, $p = 0.9$). The bar graph shows *BDNF* Val[66]Met effects (Met[66] carriers in black < Val/Val in white) on DA release during placebo administration in the right NAc. Data are expressed as means ± SEM. Significant differences between genotype groups are marked with asterisks (***$p < 0.001$).

Val[66]Met (rs6265) effect on pain ratings and placebo effectiveness

At a psychophysical level, we found no significant, direct effect of *BDNF* Val[66]Met on pain ratings during or immediately after the pain challenge ($n = 49$, average VAS scores acquired every 15 s during the challenge or MPQ scores). The volume of hypertonic

saline to achieve average target pain ratings, a measure of pain sensitivity, was also not different between genotype or sex groups, nor was the ratio between VAS and volume infused. During the introduction of the placebo, we found no effect of *BDNF* Val[66]Met on psychophysical placebo responses.

Discussion

The present study examined the effects of *BDNF* Val[66]Met on basal ganglia DA receptor availability and responses to the anticipation of monetary gains and losses, as well as psychophysical and DA responses to a pain stressor, in the absence and presence of a placebo with analgesic properties. Compared with Val/Val homozygotes, *BDNF* Met[66] carriers showed increased BOLD responses during anticipation of monetary losses (but not gains) in the VTA-NAc-mPFC circuit and greater DA release in the NAc during a pain challenge. Sustained pain-induced DA release in the NAc was positively correlated with subjective pain ratings. Moreover, a mediation analysis confirmed that *BDNF* Val[66]Met effects on the subjective pain experience were mediated by the magnitude of DA release in the NAc during the pain challenge. Conversely, compared with Val/Val homozygotes, an overall reduction in DA neurotransmission was observed in Met[66] carriers during placebo-induced DA release in the NAc. Finally, *BDNF* Met[66] carriers also showed increased $D_{2/3}$ BP_{ND} at baseline compared with Val/Val homozygotes.

Evidence from animal (Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999) and human *in vivo* imaging studies have shown that DA neurons in the mesolimbic system, especially in the ventral striatum, increase their firing and DA release in response to reward (Wise, 2004; Schultz, 2006; Peciña et al., 2012), but also to psychological (Pruessner et al., 2004; Montgomery et al., 2006; Soliman et al., 2008; Kobiella et al., 2010), physical (Scott et al., 2006; Scott et al., 2008; Peciña et al., 2012), or pharmacological stressors (Treadway et al., 2012). BDNF plays a key role in the survival and function of DA neurons, including neurons in the VTA that project to the NAc. Animal studies have demonstrated that the rodent striatum is rich in BDNF protein supplied from afferent midbrain DA and corticostriatal glutamate neurons (Altar et al., 1992; Conner et al., 1997; Kolbeck et al., 1999). Consistent with the neurotrophic role of BDNF in DA neurons in animal models, we observed a significant effect of *BDNF* Val[66]Met on DA receptor availability, in which *BDNF* Met[66] carriers showed greater baseline $D_{2/3}$ BP_{ND} than Val/Val homozygotes. This is likely a consequence of an upregulation of this receptor sites in the presence of chronic lower DA tone, as would be expected by the lower BDNF function in this group.

Exogenous BDNF has been shown to promote the survival and differentiation of cultured DA neurons (Hyman et al., 1991; Spina et al., 1992) and is associated with an overall increase in tyrosine hydroxylase activity, DA release, DA transporter uptake capacity, and DA tissue content (Hyman et al., 1991; Knüsel and Hefti, 1991; Beck et al., 1993; Zhou et al., 1994; Blöchl and Sirrenberg, 1996; Hoover et al., 2007). Consistent with this crucial role of BDNF in regulating DA release, we observed that the *BDNF* Val[66]Met polymorphism had effects on regional activation during the anticipation of monetary loss and pain- and placebo-induced DA release. However, *BDNF* Met[66] carriers, who had lower levels of BDNF and potentially lower activity-dependent release of DA, had a selective increase in brain response to the anticipation of monetary losses (but not rewards) in the VTA-NAc-mPFC cortex and DA release in the NAc during the pain challenge (the latter correlating with subjective pain ratings), but an overall reduction in DA activity in the bilateral NAc during placebo administration compared with *Val* homozygotes. These results suggest a valence-selective effect of BDNF in regulating DA-mediated aversive stimuli. During the anticipation of monetary losses, greater BOLD responses were also observed in the Hipp in Met[66] carriers, a region involved in the regulation of DA neural activity via a Hipp-NAc-ventral pallidum-VTA pathway (Floresco et al., 2001), and responses to aversive stimuli (Valenti et al., 2011). Consistent with the data presented here, *BDNF* Met[66] has been robustly associated with impaired fear extinction learning and increased risk for anxiety disorders (Soliman et al., 2010).

This pattern of greater BOLD responses to the anticipation of monetary loss, but no effect during the anticipation of monetary gain, as well as increases in stress-induced DA release and a blunted DA response during positive expectations (i.e., of pain relief) seems to point to a vulnerability phenotype. This vulnerability phenotype is in agreement with associations between low levels of *BDNF* and the development of depressive-like anhedonic responses in animal stress models (Duman and Monteggia, 2006) and persistent suppression of cocaine and saccharine reward after intermittent social stress in mice (Miczek et al., 2011). These findings might also explain the neurobiology underlying the frequent comorbidity between mood and substance abuse disorders (Brady and Sinha, 2005). On the contrary, animal models of stress have suggested that preventing BDNF signaling to the NAc may be a key molecular mechanism of stress resiliency (Berton et al., 2006; Krishnan et al., 2007). Susceptibility to an avoidant phenotype was induced by upregulation of VTA neuronal activity, which resulted in increased BDNF signaling within the NAc (Berton et al., 2006). Moreover, although Val/Val and Met/Met mice showed comparable baseline responses in forced swim and sucrose preference tests (Chen et al., 2006), a differential phenotype became evident after chronic social defeat: whereas Val/Val mice demonstrated a significant reduction in social interaction after defeat, Met/Met mice displayed an unsusceptible phenotype (Krishnan et al., 2007). As acknowledged by the investigators (Russo and Nestler, 2013), an important caveat regarding that work is that most chronic stress paradigms in *BDNF* knock-out mice have relied solely on data acquired in males (Berton et al., 2006; Krishnan et al., 2007). Data in female mice suggests that they may have increased stress vulnerability after chronic unpredictable stress (CUS; Autry et al., 2009). In the present study, loss of *BDNF* in female mice was associated with increases in anxiety and depression-like behaviors after CUS compared with wild-type littermates, effects that were not observed in males. It has also been suggested that organizational differences in the development of reward-related neural circuits might predispose women to depression (Blehar, 2006). Further studies would have to carefully consider a potential gene × sex interaction modulating stress resiliency mechanisms in humans. The data presented here are not adequately powered to examine those interactions.

The present work provides initial evidence regarding the effect of *BDNF* Val[66]Met polymorphism modulating DA-mediated stress and reward responses in the human striatum. We identify a potential vulnerability phenotype in *BDNF* Met[66] carriers defined by: (1) greater BP_{ND} in the NAc at baseline than *Val* homozygotes, suggesting chronic lower DA tone; (2) greater BOLD responses in the VTA-NAc-mPFC circuit during the anticipation of monetary losses, but not during gains; and (3) greater DA release in the NAc during a pain stressor, but a blunted DA response to the administration of a placebo with potential analgesic properties. Interindividual variability within the *BDNF* human

gene therefore appears to be involved in the neuroplastic changes that follow responses to stress and might be involved in the vulnerability and recovery from stressful experiences, potentially affecting the pathophysiology and chronicity of stress related disorders.

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