Imbalanced Neural Responsivity to Risk and Reward Indicates Stress Vulnerability in Humans

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Trauma-related psychopathology has been associated with an intense emotional reaction to stressful event. Emotional responses have evolved to signal the presence of risks to be avoided or of rewards to be approached in the environment. Thus, individuals' sensitivity to signals of risk and reward may affect the level of stress vulnerability. Stress, however, can modify these sensitivities as well. In the current functional magnetic resonance imaging (fMRI) study, we prospectively probed the neural correlates of such sensitivities in 24 healthy soldiers by using an interactive game that encompasses risky and rewarding intervals both pre-exposure and post-exposure to stressful military service. As expected, risky and rewarding intervals elicited selective responses in the amygdala and nucleus accumbens (Nacc), respectively. Furthermore, increased post-traumatic stress disorder symptoms post-exposure (i.e., stress vulnerability) corresponded to greater amygdala's response to risk both pre-exposure and post-exposure and to decreased NAcc response to reward only post-exposure. By combining these regional responsivities post-exposure, we accurately identified all the most vulnerable soldiers. Imbalanced neural responsivity to risk and reward following exposure to stress may therefore constitute a marker for stress vulnerability. Such identification of vulnerability biomarkers can aid future diagnostic and therapeutic efforts by allowing early detection of vulnerability as well as follow up on patient's treatment progression.

Keywords: amygdala, fMRI, nucleus accumbens, predisposition, prospective study

Introduction

Whether a person will suffer from chronic psychopathologies following a life threatening stressful event is crucially associated with the intensity of their initial emotional reaction to the situation (Creamer et al. 2005). Emotional reactions, as such, may have evolved to signal the risk of potential harms to be avoided or of the presence of rewards to be approached in the environment (Schwarz 2000; Cardinal et al. 2002). Taken together, this implies that individual differences in the sensitivity to motivational cues that signal risk and/or reward may be a mechanism that explains the tremendous variability in humans' response to stress, through their role in modulating emotional reactions. Therefore, reliable neural characterization of these sensitivities may point to one's vulnerability to psychopathology either prior to or following exposure to a traumatic event.

A vast amount of research have supported the notion that the amygdala and nucleus accumbens (Nacc) are 2 central nodes within the neural circuits that mediate the sensitivity to signals of potential harm and reward, respectively (Davis 1992; Depue and Collins 1999; Zald 2003; Hariri 2009; Haber and Knutson 2010). Specifically, amygdala's activation is thought to signal the presence of potential danger in the environment and thus to trigger the "fight-or-flight" response (LeDoux 2000). On the other hand, dopamine release in the Nacc is considered a hallmark of the neural reward system signaling for positive arousal (Knutson and Gibbs 2007) to enhance approach motivation (Smillie 2008).

Patients of common stress-related psychopathologies such as post-traumatic stress disorder (PTSD) and depression where shown to exhibit increased amygdala response to a variety of aversive stimuli (Fales et al. 2008; Liberzon and Sripada 2008; Shin and Liberzon 2010) and decreased Nacc response to reward (Sailer et al. 2008; Elman et al. 2009; Pizzagalli et al. 2009). Retrospective neuroimaging studies, however, cannot attribute neuronal indicators of functional abnormalities to acquired versus predisposed stress vulnerabilities (Shin and Liberzon 2010). Indeed, both animal and human studies found that stress can modify the functional properties of those exact brain regions (Cabib and Puglisi-Allegra 1996; Kavushansky and Richter-Levin 2006; Admon et al. 2009; van Wingen et al. 2011). Thus, for a reliable characterization and identification of stress vulnerability, we must assume that activation in brain regions that mediate responsivity to signals of risk and reward not only influences the individual's emotional response to environmental challenges but that environmental factors, and especially stress, can have a modifying effect on them too (Hariri 2009).

The aim of this prospective functional magnetic resonance imaging (fMRI) study, therefore, is to disentangle acquired from predisposed stress vulnerabilities in humans based on neuronal measurements of the individual's sensitivity to signals of risk and reward. In order to attain this goal, we used an interactive naturalistic game that encompasses distinct time intervals of both risky anticipation to punishment and receiving unpredictable reward (Fig. 1). Prior work from our lab has already shown that these intervals evoke selective responses in the amygdala and Nacc, respectively, as well as increased skin conductance response during risky anticipation (Kahn et al. 2002; Assaf et al. 2009). Furthermore, these intervals probe the exact dysfunctional processes of stress-related patients since it seems that in relation to reward, their abnormal responses arise in the outcome consumption phase, while in relation to aversive stimuli, their abnormality is most prominent during the expectancy
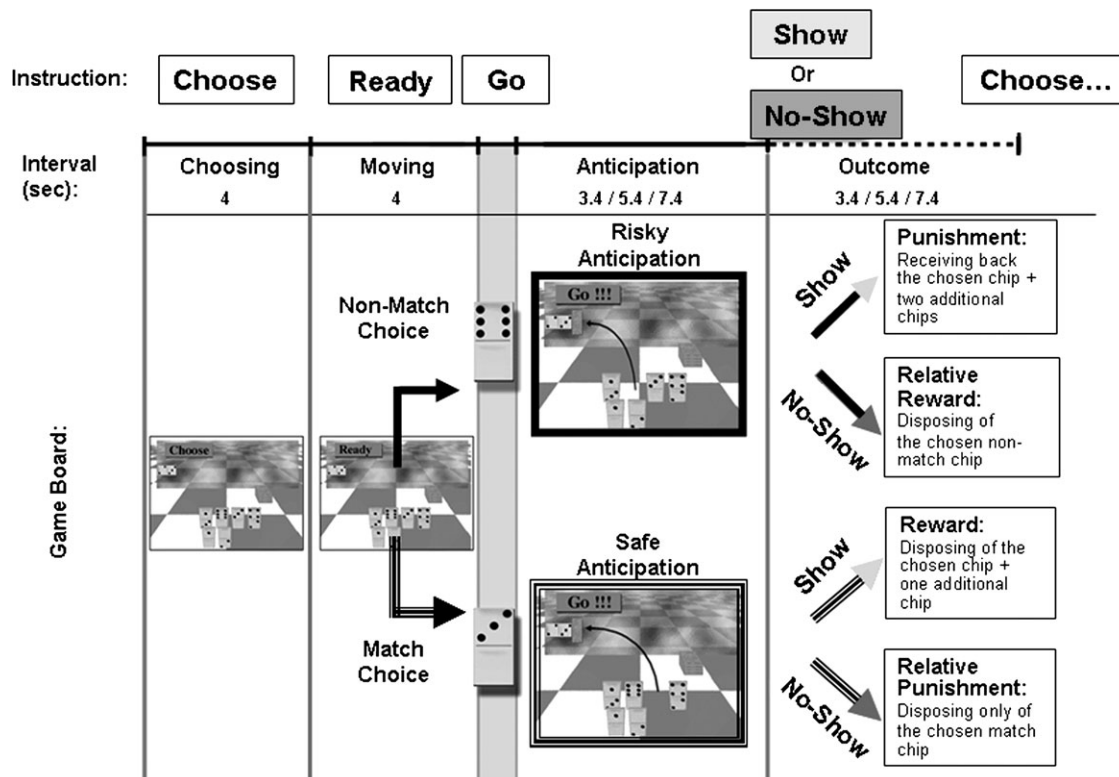
Figure 1. fMRI paradigm. Each round of the game is composed of 4 intervals: the player chooses which chip to play next (first interval: "Choose"; 4 s), moves the cursor to the chosen chip, and places it face down adjacent to the master chip (second interval: "Ready" and "Go"; 4 s). The player then waits for the opponent's response (third interval: "Anticipation"; jittered randomly to 3.4, 5.4, or 7.4 s) and sees whether the opponent challenges this choice by uncovering the chosen chip or not (fourth interval: "Outcome"; jittered randomly to 3.4, 5.4, or 7.4). Player's choices and opponent's responses are interactively determined by the flow of the game round after round, creating a natural progression of a game situation that lasts 4 min or until the player wins. Each player played consecutively for 14 min at each testing point (average ± standard error of the mean number of games 4.23 ± 0.06).

that precedes the potentially negative outcome. This fMRI protocol was used in a prospective design in which we followed a group of 24 a priori healthy soldiers throughout their combative mandatory military service as paramedics in the Israeli Defense Force (IDF). Specifically, the study's first time point was immediately before military draft, while participants were still civilians that are already recruited to the combat paramedics unit and the second time point was 18 months later, while participants were serving as combat paramedics in front line IDF military units. Notably, this exact time period of combative military service was previously shown by us to include exposure to multiple real-life stressful events (Admon et al. 2009). Thus, we were able to compare in here the individual behavioral and neuronal responses to risk and reward pre-exposure versus post-exposure to such stress.

We expected that individuals' vulnerability to stress would be manifested by the combination of high amygdala responsivity to risk and low Nacc responsivity to reward. Furthermore, based on our previous findings (Admon et al. 2009), we hypothesized that high amygdala responsivity to risk would already be evident prior to the stressful encounter in vulnerable individuals as predisposition.

Materials and Methods

Participants
The study group comprised 24 healthy 18-year-old soldiers (12 males) recently drafted to mandatory military service to serve as combat

paramedics in the IDF. In order to be drafted for an elite unit of the IDF such as combat paramedics, a recruit must first pass a series of mental and physical tests as well as an examination by both a physician and a psychiatrist. These processes are meant to rule out, among other things, any history of psychiatric or neurological disorders and any past or present use of psychoactive drugs. Since at the first time point of this study, our participants were already recruited for the combat paramedics unit and passed all of the above selection criteria, we can rule out the existence of any of those factors before entering the study. Furthermore, at the study's first time point, participants were asked by us to fulfill a questioner with several open questions regarding life history of traumatic and/or significant experiences and illnesses. These included specific questions regarding any sever mental and/or physical illnesses and/or hospitalizations for the participant and his close circle family, as well as an open question in which the participants were asked to describe any traumatic and/or significant experience that occurred throughout their 18 years of civilian life prior to the military draft and its emotional effect on them. Only individuals that reported no history of psychiatric disorders for them and their families and no traumatic experiences before recruitment were selected to participate in the study. Finally, all participants provided written informed consent approved by the Tel Aviv Sourasky Medical Center Ethics Committee.

Stress Symptom Evaluation
PTSD and depression are common psychopathologies that may develop following exposure to stress. Therefore, we used the Post-traumatic Stress Diagnostic Scale (PDS) (Foa et al. 1997) and the Beck Depression Inventory (BDI) (Beck et al. 1961) questionnaires to yield quantified severity measures of PTSD-related (PDS) and/or depression-related symptoms (BDI). The PDS also contains an open question regarding the type and frequency of stressful experiences. We instructed the participants to complete the questionnaire in regard to the past 18 months,

thus, we were able to evaluate post-exposure both the type and the frequency of the individual experiences endured during their military service as well as quantify the severity of symptoms that were developed following such experiences (Table 1).

fMRI Paradigm

Participants played a 2 players competitive Domino game in which the scanned subject is the player, while a computer randomly generates the opponent's responses in a predetermined pattern to allow a balanced design. Players, however, are told that the opponent is the experimenter and that their choices can increase their chances of winning. At the beginning of each game, 12 random domino chips are assigned to the player and are shown on the bottom part of the board, while one master domino chip, which is constant throughout the game, appears on the top left corner of the board. In each round of the game, the player chooses one chip, places it face down adjacent to the master chip, and then waits for the opponent's response (i.e., anticipation) to see whether the opponent challenges this choice by uncovering the chosen chip or not (i.e., outcome). The player wins the game if he is able to successfully dispose of all 12 chips within 4 min. Each assigned chip can either match the master chip (have one of the master chip's numbers) or not. Since the master chip is constant throughout the game, it is only possible to win by choosing both matching and nonmatching chips. In the game context, matching chips are considered "safe" moves since they are associated with rewards and nonmatching chips are considered "risky" moves, since they are associated with punishments if uncovered. Accordingly, based on the player's choice, there are 2 possible anticipation periods: risky anticipation following a nonmatch choice or safe anticipation following a match choice. In addition, based on the player's choice and opponent's response, there are 4 possible consequences per game round (i.e., "outcome" possibilities): 1) Show of a nonmatch chip: the choice of a nonmatch chip is exposed and the player is punished by receiving back the selected chip plus 2 additional chips from the deck. 2) No show of a nonmatch chip: the choice of a nonmatch chip remains unexposed and only the selected chip is disposed of, so the player is relatively rewarded as he gets away with a nonmatch choice. 3) Show of match chip: the choice of a match chip is exposed and the player is rewarded by disposal of the selected chip and one additional random chip from the game board. 4) No show of a match chip: the choice of a match chip is not exposed and only the selected match chip is disposed of, so the player is relatively punished as he could have disposed of a nonmatch chip instead. For more details of the game, see Figure 1 as well as Kahn et al. (2002) and Assaf et al. (2009).

Behavioral Analysis of the Game

To characterize the player's behavioral choices during the game a risky choice index was defined as the ratio between the number of game rounds in which a player choose a safe match chip over the total number of their choices throughout the entire game. This index represents a nonbiased choice when equal to 0.5 (exactly half of the choices were matching chips), a choice bias for safe matching chips when smaller than 0.5 or for risky nonmatching chips when greater than 0.5.

fMRI Data Acquisition

Brain scanning was performed by a 3T GE scanner with a standard head coil. fMRI was performed with gradient echo-planar imaging sequence of functional T_1^*-weighted images (time repetition /time echo/flip angle = 2500 ms/35 ms/90°, with field of view 20 × 20 cm, matrix size 64 × 64) divided into 44 axial slices (thickness: 3 mm with no gap) covering the entire brain.

fMRI Data Preprocessing

Statistical Parametric Mapping software package, SPM5 (Welcome Department of Imaging Neuroscience, London, UK) and Marsbar toolbox were used with Matlab 7.0.4 (MathWork, Natick, MA). Preprocessing of functional scans included slice timing and head movement correction, normalizing the images to Montreal Neurological Institute (MNI) space and finally spatially smoothing the data (full-width at half-maximum: 6 mm). In addition, a set of harmonics was used to account for low frequency noise in the data (1/128 Hz), and the first 6 images of each functional scan were rejected to allow for T_1^* equilibration effects.

Whole-Brain Analysis

The size of the effect for each condition for each participant was computed by a general linear model (GLM) that included the participant's 2 scans of pre-exposure and post-exposure. However, regressors of each condition were modeled separately for each of the 2 scans. GLM regressors of the Anticipation interval were sorted according to the player's choice of 1) matching or 2) nonmatching chips and of Outcome interval according to the player's choice and the opponent's response to derive the 4 conditions of 1) show match, 2) show nonmatch, 3) no show match, and 4) no show nonmatch (see Fig. 1). Importantly, events in which the player has no real ability to choose the level of risk are rare yet possible (i.e., if only matching chips or only nonmatching chips remained to choose from). When such events occur, we disregarded them by modulating the entire specific game round as a separate "Don't-care" regressor in our GLM (less then 2% of our data). Next, whole-brain individual statistical parametric maps were separately calculated for the 2 a priori defined contrasts of interest: risky anticipation > safe anticipation (waiting for the opponent's response following nonmatch vs. match choices) and receiving rewarding outcome > receiving punishing outcome (opponent's show following match choice and no show following a nonmatch choice vs. opponent's show following a nonmatch choice and no show following a match choice). Individual contrast parameter estimates were then used in a second level, random effects group analyses with SPM5 2 sample T-test analysis in which the effects of each time point were entered as a separate group and the contrast between the groups (i.e., time points) was +1 +1 thus not contrasting between the time points but combining them. This analysis was done separately for each

Table 1

Stressful encounters during military service and consequent rise in PTSD-related and/or depression-related symptoms

Number	Gender	Number of stressful encounters	PTSD-related symptoms post-exposure	Depression-related symptoms post-exposure
1	Male	10	0	1
2	Male	3	3	5
3	Male	3	2	6
4	Male	2	0	1
5	Male	20	6	8
6	Male	2	2	1
7	Male	13	**16**	11
8	Male	2	1	3
9	Male	5	0	3
10	Male	13	4	7
11	Male	18	0	1
12	Male	10	**15**	5
13	Female	8	9	2
14	Female	5	**17**	**19**
15	Female	13	7	0
16	Female	5	6	3
17	Female	10	3	3
18	Female	20	**15**	5
19	Female	10	10	9
20	Female	13	1	4
21	Female	10	0	1
22	Female	7	2	6
23	Female	12	2	0
24	Female	13	6	4

Note: The number of stressful encounters and level of PTSD-related symptoms post-exposure is based on the PDS questionnaire (Foa et al. 1997). The level of depression-related symptoms post-exposure is based on the BDI questionnaire (Beck et al. 1961). Bold numbers represent a level of symptoms which is above the criteria for mild PTSD and/or mild depression. Soldiers that developed enough symptoms to reach either of the questionnaires cutoff are therefore consider as the most vulnerable. None of the soldiers in the study group suffered from any PTSD- and/or depression-related symptoms pre-exposure based on the PDS or BDI questionnaires (data not shown).

of our 2 a priori defined contrasts. Significance level was set at $P < 0.05$ FDR corrected.

Region of Interest Analysis

Regions of interest (ROIs) were identified functionally from a group contrast that combined the effects of both time points together in a single whole-brain analysis (amygdala from risky anticipation and Nacc from receiving rewarding outcome) and verified anatomically according to MNI coordinates. Next, we extracted participants' activations from each ROI separately for each time point by averaging for all the voxels in the ROI the beta weights of a specific contrast in a specific time point. Notably, ROI identification and selection was "blind" to the level of stress vulnerability (i.e., post-exposure PTSD-related symptoms), thus avoiding any statistical bias between whole-brain and ROI analyses (Vul et al. 2009).

Statistical Analysis

In order to compare the behavioral risky choice index of the whole-group pre-exposure versus post-exposure (Fig. 2A), we used paired T-tests. Due to the discrete nature of our main outcome measure, we used Poisson regression, which is the most suitable approach for count data inferences (McCullagh and Nelder 1989). Specifically, Poisson regression was used to investigate the individual relation between post-exposure PTSD-related symptoms to behavioral risky choice index pre-exposure and post-exposure (Fig. 2B) and ROI activations pre-exposure and post-exposure (Fig. 3B). Equality of effects pre-exposure versus post-exposure was tested using a linear contrast on the regression coefficients. The added value of considering both amygdala

Figure 2. Behavioral manifestations of stress vulnerability. A risky choice index (see Materials and Methods) was calculated for each player during scanning at each time point of pre-exposure and post-exposure (gray and black, respectively). (A) Bar graphs representing the group average risky choice index at each time point. Note the decrease in this index post-exposure, indicating that the soldiers choose fewer risky nonmatch chips with harming prospect during the game post-exposure relative to pre-exposure ($T_{23} = 2.17$; $P = 0.04$). (B) Scatter plot and regression lines showing that the more post-exposure PTSD-related symptoms, the less often soldiers choose risky nonmatch chips during the game post-exposure ($\beta = -5.36$; $P < 0.001$) but not pre-exposure ($\beta = 0.2$; $P = 0.87$) ($N = 24$; error bars ± standard error of the mean).

and Nacc activation post-exposure versus considering each of them separately in explaining stress vulnerability was demonstrated by multiple regression analysis (Fig. 4). Finally, all above analyses were performed while controlling for the individual frequency of stressful encounters (see Table 1) and remained significant after outlier removal (i.e., data points with Deviance above 3).
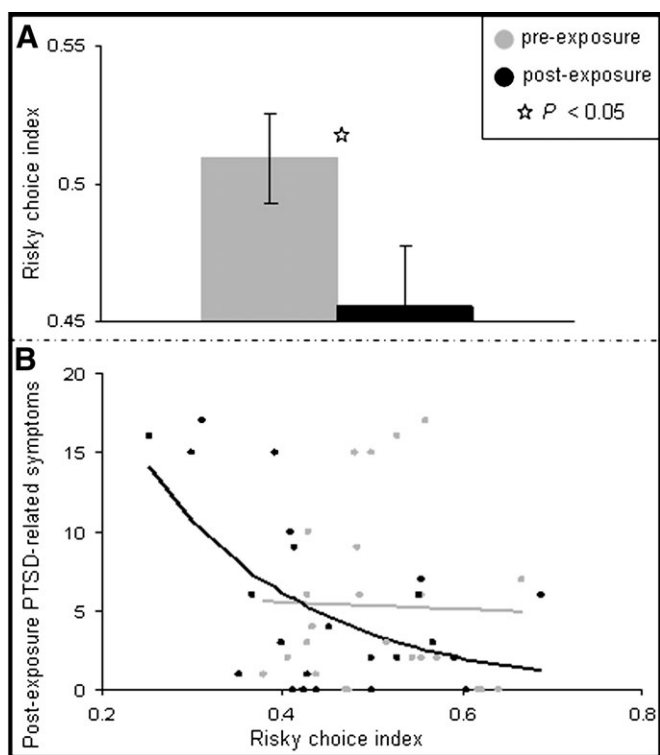
Results

Individual Stress Vulnerability

Prior to their military draft, none of the soldiers in the study group suffered from any PTSD- and/or depression-related symptoms based on the PDS or BDI questionnaires, respectively. During the 18 months of their military service, all of the soldiers were exposed to multiple stressful experiences, which, as a direct outcome of their shared role as combat paramedics, were highly comparable and included mostly repeated events of treating a fellow soldier with severe injury sustained during combat. Following such events, 19 soldiers (≈80% of the sample size) developed one or more PTSD-related symptoms and 22 soldiers (≈90%) developed one or more depression-related symptoms. Of note, 4 soldiers (≈15%) had developed enough symptoms to reach the PDS cutoff for mild PTSD, of which, 2 were also the only soldiers from the entire group to reach the BDI cutoff for mild depression (Table 1). These 4 soldiers can thus be considered as the most vulnerable individuals in our sample, although they stayed on duty and fully functional in their original role as combat paramedics throughout the study. A similar ratio of vulnerability has been repeatedly found in epidemiological studies following exposure to stress (Breslau 1998).

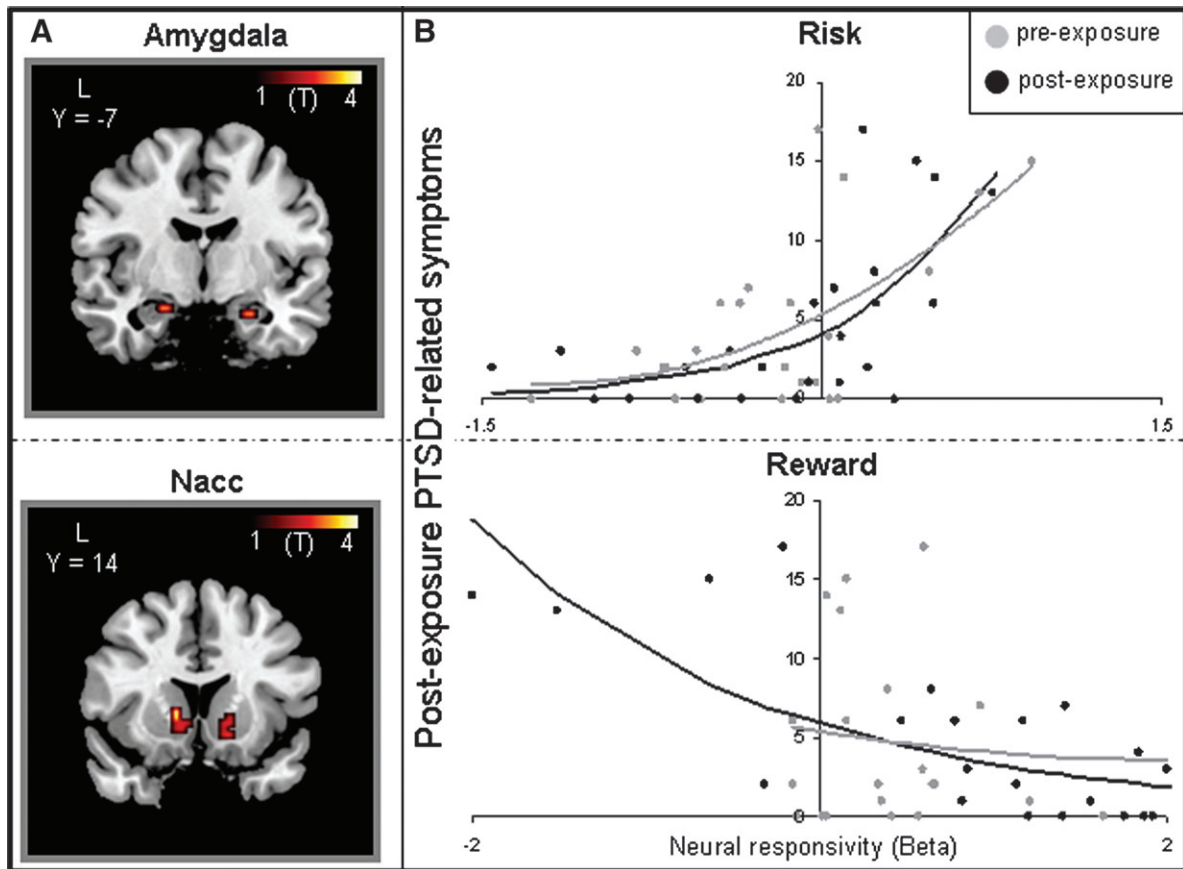### Behavioral Manifestations of Stress Vulnerability

A calculated risky choice index (see Materials and Methods) revealed a decrease in the number of risky nonmatch choices made during the game post-exposure, relative to pre-exposure ($T_{23} = 2.17$; $P = 0.04$; Fig. 2A). This could not be explained by a change in the total amount of choices the soldiers made throughout the game as it did not differ between pre-exposure to post-exposure (average ± standard deviation: 33.6 ± 2.8 choices pre-exposure and 32.3 ± 4.6 choices post-exposure; $T_{23} = 1.46$; $P = 0.17$; data not shown). At the individual level, the more PTSD-related symptoms developed post-exposure, the less often soldiers choose risky nonmatch chips during the game post-exposure ($\beta = -5.36$; $P < 0.001$) but not pre-exposure ($\beta = 0.2$; $P = 0.87$; Fig. 2B). A test for equality of coefficients supported this by showing that the relation between post-exposure PTSD-related symptoms and post-exposure risky choice index was stronger than the relation between post-exposure PTSD-related symptoms and pre-exposure risky choice index ($P < 0.001$).

Neural Responsivity to Risky Anticipation and Rewarding Outcome

A whole-brain fMRI contrast of risky anticipation versus safe anticipation revealed increased activation within several brain regions, including our predetermined ROI, the amygdala (Fig. 3A, upper panel; Table 2A). Several other brain regions responded to receiving unpredicted rewarding outcome versus receiving unpredicted punishing outcome, including our predetermined ROI, the Nacc (Fig. 3A, lower panel; Table 2B). Such increased amygdala and Nacc activations in response to

Figure 3. Neural manifestations of stress vulnerability. (A) Coronal views of brain activation obtained from whole-brain group parametric maps showing increased bilateral amygdala activation in response to risky anticipation and increased bilateral Nacc activation in response to unpredicted rewarding outcome (upper and lower panels, respectively; $P < 0.05$, FDR corrected, random effect). (B) Scatter plots and regression lines of the individual regional brain activation obtained from pre-exposure (gray dots) or post-exposure (black dots) and the individual level of post-exposure PTSD-related symptoms. Greater amygdala activation in response to risk both pre-exposure and post-exposure is related to more PTSD-related symptoms (upper panel; pre-exposure: $\beta = 1.34$, $P < 0.001$; post-exposure: $\beta = 1.65$, $P < 0.001$), while reduced Nacc activation in response to reward post-exposure is related to more PTSD-related symptoms, a relation which was not significant pre-exposure (lower panel; pre-exposure: $\beta = -0.18$, $P = 0.17$; post-exposure: $\beta = -0.57$, $P < 0.001$) ($N = 24$; error bars $\pm$ standard error of the mean).
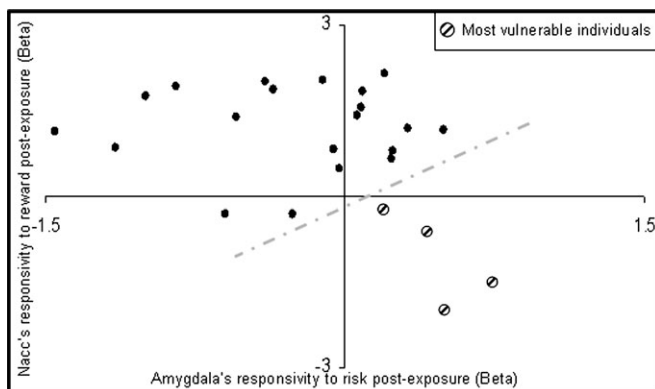
Figure 4. Neural responsivity to risk and reward indicates stress vulnerability. Scatter plot showing amygdala's responsivity to risk post-exposure (x-axis) in relation to Nacc's responsivity to reward post-exposure (y-axis). Thus, each point on the graph represents the combined measurement for an individual's responsivity to risk and reward post-exposure to stress. The most vulnerable individuals are marked by striped circles. Note that only a combined measure can achieve an accurate and complete 100% separation of these vulnerable individuals from the rest of group (striped line).

risk and reward, respectively, are consistent with previous imaging studies that used this specific paradigm (Kahn et al. 2002; Assaf et al. 2009). For both the amygdala and the Nacc,

we received bilateral activations. Following the lack of significant difference between left and right activations for both ROIs and at both time points (amygdala pre-exposure: $T_{23} = -0.86$, $P = 0.40$; amygdala post-exposure: $T_{23} = 0.38$, $P = 0.71$; Nacc pre-exposure: $T_{23} = 1.10$, $P = 0.28$; Nacc post-exposure: $T_{23} = -0.35$, $P = 0.73$; data not shown), we used the averaged bilateral activation as a single measure for ROI activation in all furtherer analyses.

Neural Manifestations of Stress Vulnerability

While examining the individual relation between post-exposure PTSD-related symptoms and ROI activations pre-exposure and post-exposure, we found that greater amygdala activation in response to risky anticipation both pre-exposure and post-exposure is related to more post-exposure PTSD-related symptoms (pre-exposure: $\beta = 1.34$, $P < 0.001$; post-exposure: $\beta = 1.65$, $P < 0.001$; no significant difference between the coefficients [$P = 0.27$]; Fig. 3B, upper panel). In the Nacc, reduced activation in response to rewarding outcome post-exposure was related to more post-exposure PTSD-related symptoms, an association which was not significant pre-exposure (pre-exposure: $\beta = -0.18$, $P = 0.17$; post-exposure: $\beta = -0.57$, $P < 0.001$; significant difference between the coefficients [$P < 0.001$]; Fig. 3B, lower panel). In a more

Table 2

Peak of activations obtained from a whole-brain contrast that combined both time points' response to risky anticipation and to receiving unpredicted rewarding outcome

Region	Cluster (#voxels)	Peak voxel (x; y; z)	Z value	P value (FDR corrected)	Relation to post-exposure PTSD-related symptoms (β; P)	
					Activation pre-exposure	Activation post-exposure
A. Response to risky anticipation (Nonmatch > Match)						
R. caudate	11	9; 0; 12	4.25	0.03	−0.45; 0.06	−0.34; 0.16
Superior frontal gyrus (BA 6)	11	3; 28; 63	4.14	0.03	−0.2; 0.12	−0.45; 0.07
L. amygdala	4	−25; −6; −21	3.75	0.04	1.34; <0.001	1.65; <0.001
R. amygdala	4	22; −6; −18	3.60			
B. Response to rewarding outcome (Show Match + No Show Nonmatch > Show Nonmatch + No Show Match)						
R. ventral striatum (Nacc)	14	9; 16; −3	4.27	0.03	−0.18; 0.17	−0.57; <0.001
L. ventral striatum (Nacc)	15	−16; 13; −9	4.22	0.03		
R. anterior cingulate cortex (BA 10)	7	6; 47; 15	3.84	0.05	−0.02; 0.77	−0.14; 0.2
L. precuneus (BA 7)	5	−3; −75; 48	3.71	0.05	−0.32; 0.23	−0.22; 0.2
L. inferior parietal lobe (BA 40)	4	−47; −38; 45	3.59	0.05	0.1; 0.26	−0.03; 0.57

Note: Localization is based on MNI criteria. Estimated level of activation is described by Z score and P values. $P < 0.05$, FDR corrected, random effect. L, left; R, right; BA, Brodmann area. $N = 24$.

explorative approach, we also looked for the relation between the individuals' level of post-exposure PTSD-related symptoms and activations in all other regions that displayed increased activations at our whole-brain group contrasts. Notably, the activations in none of those additional regions, either pre-exposure or post-exposure to stress was correlated with symptom severity (Table 2A and B).

Imbalanced Neural Responsivity to Risk and Reward Indicates Stress Vulnerability

In order to characterize the post-exposure neural marker of stress vulnerability, we plotted the individuals' amygdala responsivity to risk and Nacc responsivity to reward post-exposure. This allowed us to estimate the ability to identify the most vulnerable individuals based on each neural feature individually and combined. Figure 4 shows that both amygdala responsivity to risky anticipation and Nacc responsivity to rewarding outcome can separately detect stress vulnerability to some extent. However, it was only possible to achieve an accurate and complete 100% identification of the most vulnerable individuals by combining information on individual neural responsivity to both risk and reward post-exposure (striped line). This integrative finding is further supported by multiple regression analysis showing that considering both amygdala and Nacc activation post-exposure versus considering each of them separately significantly contributes to our ability to explain the variability in the level of post-exposure PTSD-related symptoms ($P_{\text{amygdala removal}} < 0.001$; $P_{\text{Nacc removal}} < 0.001$).

Discussion

With the use of an interactive competitive game, we demonstrated increased amygdala and Nacc activation in response to risky anticipation and rewarding outcome, respectively, in accordance with previous suggestion about the functions of those brain regions (Davis 1992; Depue and Collins 1999; Zald 2003; Hariri 2009; Haber and Knutson 2010). By implementing this paradigm prospectively both pre-exposure and post-exposure to stressful military service, we were able to show in here that stress vulnerability in humans is related both to increased amygdala response to risk as well as to decreased Nacc response to reward.

Corresponding to our previous brain imaging findings (Admon et al. 2009), the most vulnerable soldiers exhibited increased amygdala response during risky anticipation to

punishment, both pre-exposure and post-exposure to stress. The repeated demonstrations of increased amygdala response to aversive stimuli in stress psychopathology (Liberzon and Sripada 2008; Shin and Liberzon 2010), therefore, seem to reflect a predisposing risk factor that increases vulnerability to stress. Indeed, amygdala injuries sustained during combat were found to protect against the later development of PTSD in combat veterans (Koenigs et al. 2008). This demonstrated relationship between excessive amygdala responsivity to risk and predisposed stress vulnerability may be explained by the well-documented role of the amygdala in modulating fear response (Davis 1992), possibly via recruitment of the brainstem arousal system (Cardinal et al. 2002). Thus, a priori high amygdala activation may lead to amplified fear-related arousal when facing a potential harm (Haas et al. 2007). Such negative perceptions of what might happen can elicit exaggerated anxiety (Butler and Mathews 1987), placing greater demand on these individuals' capacity to effectively cope with the stressful experience (McNally 2003). This is consistent with cognitive theories of anxiety, which suggest that expectancy processes play an important role in the production and maintenance of anxiety pathology (Beck et al. 1985).

Stress vulnerability was also characterized by diminished Nacc response to rewarding outcome but only post-exposure. It is possible, therefore, that the demonstrated decreased Nacc response to positive stimuli among patients of stress-related disorders (Sailer et al. 2008; Elman et al. 2009) is a consequence of the maladaptive response to stress more than a predisposing risk factor. Such diminished Nacc response to rewarding outcome may then lead to lower satisfaction while receiving the reward as it loses its saliency (Sailer et al. 2008). Animal findings support this possibility by showing that unpredictable stress can cause a dysfunction within Nacc's dopaminergic tracts, thus leading to an alternation of the rewarding properties of the stimuli and therefore to a decrease in its hedonic value (Cabib and Puglisi-Allegra 1996). Intriguingly, Nacc-related impaired reward processing was described as a characterizing feature of depressive patients (Pizzagalli et al. 2009). Considering the extremely high percentage of comorbidity that is shared by PTSD and depression (Bleich et al. 1997), we can speculate in here that it is the maladaptive reduction in Nacc reward responsivity that links these 2 common stress-related disorders together.

Following exposure to stress neural responsivity to either risk or reward alone was unable to independently separate the

most vulnerable soldiers from the rest of the group. Thus, along with prior suggestions that the behavioral decision to approach or to avoid a stimuli in the environment is influenced from the activation of those 2 opposing motivational tendencies (McNaughton and Corr 2004); we suggest that this behavioral output is indicated in humans at the neuronal level by the combined responsivity of the Nacc and amygdala, respectively. This suggests that stress-induced diminished Nacc response to reward combined with predisposed high amygdala's responsivity to potential harm may represent an underlying neural mechanism for vulnerability to stress psychopathology in humans. Such a breach of balance between 2 distinct neural nodes that mediate complimentary motivations can lead to supremacy of avoidance behavior with anhedonic affective status; both are major debilitating symptoms of stress-related psychopathology. In support of this hypothesis, the amygdala and Nacc are known to be anatomically connected (Petrovich et al. 1996) and more importantly, negatively modulate each other's response to stress (Stevenson and Gratton 2003). Our behavioral finding also supports this hypothesis as post-exposure soldiers choose fewer times the risky nonmatch choice during the game, which suggests that the risk component had higher weight then the rewarding one in deciding how to behave. The fact that such behavioral pattern positively correlated with the level of symptoms post-exposure provides additional support for our claim that imbalanced responsivity to risk and reward is associated with stress vulnerability.

The individual level of vulnerability to stress is clearly a complex phenomena determined by genetic, developmental, biological, and psychological factors as well as their interaction. For example, the current study cannot distinguish whether the origins of the predisposing increased amygdala response to risk are genetically or environmentally determined. Furthermore, our definition of vulnerability is based on excessive symptoms following stress, thus should by validated with regard to a formal diagnoses. Finally, the amygdala and Nacc were shown to respond to a wide range of stimuli including both positive and negative ones (Zald 2003; Pruessner et al. 2004; Delgado et al. 2008; Morrison and Salzman 2010). Therefore, our neural model of vulnerability is clearly a simplification of human's response to motivational signals, which probably involves multiple interacting brain regions. Nevertheless, our results may be seen as an example of how identification of vulnerability biomarkers could aid future diagnostic and therapeutic efforts by allowing early detection of vulnerability as well as follow up on patient's treatment progression. To date, all such processes are still solely based on the patient's self-reported symptomatic descriptions and thus can only be established when patients reach a full-blown chronic stage of the disorder, a delay which severely hampers prognosis.

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