The Origin of Physiological and Functional Brain Abnormalities During Fear Conditioning in Identical Twins Discordant for PTSD and Trauma Exposure

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Abstract

Psychophysiological and neuroimaging research has demonstrated impaired fear conditioning responses in PTSD. However, the origin of these biological abnormalities has not yet been identified. The purpose of this study was to determine whether these psychophysiological and neural abnormalities in PTSD are familial vulnerability factors, effects of trauma exposure, or are acquired characteristics of PTSD. In this case-control twin study, monozygotic twin pairs discordant for combat exposure completed a fear conditioning and extinction paradigm. Skin conductance responses (SCR) and brain activation during the task were measured. Results indicate that a decreased ability to distinguish between safety and threat cues, as indicated by SCR, and increased activation in the left and right insula during early trials of conditioning may be familial vulnerability factors of PTSD. The origin of these abnormalities has potential clinical implications for primary and/or secondary prevention of PTSD.

Keywords: posttraumatic stress disorder, fear conditioning, skin conductance response, functional magnetic resonance imaging

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The Origin of Functional Brain Abnormalities During Fear Conditioning in Identical Twins Discordant for PTSD and Trauma Exposure

Fear is a fundamental biological response to threat that serves the important evolutionary purpose of keeping organisms safe from harm. Although fear is generally adaptive, if taken to an extreme it can be maladaptive. Abnormally elevated levels of fear when there is no actual threat can be associated with distress and anxiety (e.g., VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). This is seen in trauma-and-stressor-related disorders such as posttraumatic stress disorder (PTSD).

Posttraumatic Stress Disorder (PTSD)

PTSD is a disorder that may impact those exposed to one or more terrifying and life-threatening traumatic events (American Psychiatric Association, 2013), including warfare, motor-vehicle accidents, and physical and sexual assault. PTSD has a lifetime prevalence of 15-30% in Vietnam combat veterans (Dohrenwend, Turner, Turse, Adams, Koenen, & Marshall, 2006). In the Diagnostic and Statistical Manual of Mental Disorders (5th edition) (DSM-5), PTSD is characterized by 1) direct or indirect exposure to death, serious injury, or sexual violence; 2) re-experiencing distressing memory, nightmares, or dissociative reactions; 3) persistent avoidance of trauma reminders; 4) negative alterations in cognitions and mood; and 5) alterations in arousal and reactivity including exaggerated startle and hypervigilance (American Psychiatric Association, 2013).

Individuals with PTSD often experience heightened levels of fear and arousal even when no actual threat is present (e.g., Jovanovic, Kazama, Bachevalier, & Davis, 2012). For example, soldiers who recently returned to the United States from a tour of service in Iraq might still become fearful, aroused, and hypervigilant when they encounter the sight of a Humvee, which

had commonly predicated an explosion in Iraq. After repeated exposure to a Humvee back in the United States, the soldiers should learn that the Humvee no longer predicts any explosion. However, individuals with PTSD fail to learn this and have difficulty decreasing their fear response to the threat cue, which is no longer a threat (Wessa & Flor, 2007; Blechert, Michael, Vriends, Margraf, & Wilhelm, 2007; Milad et al., 2008; Jovanovic et al., 2010; Milad et al., 2009; Glover et al., 2011; Garfinkel et al., 2014).

Fear Conditioning

Pavlovian fear conditioning and extinction paradigm is a prominent experimental model for understanding the symptoms of PTSD. During conditioning, an association is formed such that a previously neutral stimulus called the conditioned stimulus (CS) predicts a biologically relevant stimulus called an unconditioned stimulus (UCS) and leads to a conditioned response (CR) and associative memory formation. For example, a neutral stimulus such as a Humvee (CS) predicts an explosion (UCS) and leads to fear (CR) to the Humvee.

Following conditioning, if the CS is repeatedly presented without the UCS, a new form of learning called extinction learning should occur. For example, when soldiers leave Iraq and return to the United States, the soldiers should begin to learn that the Humvee that used to predict threat, no longer does. Furthermore, after a delay of typically 24 hours, the retrieval and expression of the learned extinction memory can be assessed in a phase called extinction recall. This phase allows researchers to explore whether or not there was successful consolidation and retrieval of the associations formed during extinction learning. Specifically, researchers can understand whether or not soldiers are able to consolidate, retain, and retrieve the learned association that when in the United States, the Humvee no longer predicts an explosion.

To understand these processes of fear conditioning, rigorous laboratory experiments have been conducted in animals, healthy humans, and in clinical populations, specifically those with PTSD. In this paper, we will focus on skin conductance response (SCR) and functional magnetic resonance imaging (fMRI) findings in the conditioning phase of the fear conditioning and extinction paradigm.

Psychophysiology and Neurocircuitry of Fear Conditioning in Animals

Animal studies have set the foundation for understanding fear conditioning in healthy humans and clinical populations. Psychophysiological responses in animals are measured in percentage of time spent freezing by rodents and by fear-potentiated startle response (FPSR). Lights or tones are used as the CS and foot shock as the US. Greater freezing and FPSR are observed in rodents when the CS predicts the US (Davis, 2001). These psychophysiological responses observed during fear conditioning in animals are controlled by the autonomic nervous system, specifically the sympathetic nervous system, which is regulated by the brain. Lesion, staining, and electrical stimulation techniques are used to map the neurocircuitry involved in the behavioral changes observed during fear conditioning. It is important to assess the relationship between behavior and brain to fully understand the mechanisms associated with fear conditioning and to be able to translate these findings towards healthy humans and clinical populations for prevention and treatment.

By assessing changes in freezing and FPSR, the neurocircuitry involved in fear conditioning has been well mapped out in rodents. The sensory experience of the CS and US are processed in the thalamus and somatosensory cortex and this sensory information travels to the lateral amygdala via one of two pathways: cortical pathway or subcortical pathway (LeDoux, 2000; Wilensky, Schafe, Kristensen, & LeDoux, 2006; LeDoux & Pine, 2016). The cortical

pathway entails relaying sensory information from the sensory thalamus to the sensory cortex and hippocampus and then to the lateral amygdala. The subcortical pathway relays sensory information from the thalamus to the lateral nucleus and then to the central nuclei of the amygdala. The lateral nucleus is the gateway into the amygdala and the central nucleus is the output that controls emotional responses. Specifically, the CS-US association is formed in the lateral nucleus of the amygdala and the freezing (Blair, Schafe, Bauer, Rodrigues, & LeDoux, 2001; Pitkanen, 2000) and FPSR (Campeau & Davis, 1995) occurs via outputs from the central nucleus to the periacquaductal gray and reticulopontis caudalis in the brainstem, respectively (LeDoux, 1996). The subcortical pathway is a faster transmission route that is direct and short but it bypasses cortical processing, which can lead to deficits in producing appropriate fear response. Additionally, the dorsal prelimbic cortex of rodents is involved in the expression of conditioned fear (Burgos-Robles, Vidal-Gonzalez, & Quirk, 2009) and the hippocampus is involved in comparing novel cues to existing CS and determining if a CR is appropriate (Maren, 2013).

The animal literature provides a strong understanding of the fear neurocircuitry and has been used as a guide to understand the fear neurocircuitry in healthy humans and in clinical populations (discussed below).

Psychophysiology and Neurocircuitry of Fear Conditioning in Healthy Humans

Psychophysiological responses to fear conditioning in humans are assessed using SCR, heart rate response (HRR), and FPSR. SCR is a physiological measure of the sympathetic nervous system's response to fear or perceived threat. Electrodes placed on the hypothenar surface of the non-dominant hand are used to measure the moisture level of the skin. The relationship between SCR and the autonomic nervous system has been well established in the

human literature using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). The amygdala has been closely related to the expression of SCR during early fear conditioning (Cheng, Richards, & Helmstetter, 2007). Failure to demonstrate conditional SCR, while maintaining declarative knowledge of the conditioning procedures, has been observed in patients with amygdala lesions (Bechara et al., 1995; LaBar et al., 1998).

Fear conditioning studies have shown increased psychophysiological responses during fear conditioning in healthy humans (Cohen & Randall, 1984; Hamm & Vaitl, 1996). Successful fear conditioning is observed when SCR is higher to a CS+ (a CS that predicts shock) relative to a CS- (a different CS that never predicts shock). CS+ and CS- are usually referred to as the threat cue and safety cue, respectively. Higher HRR (Hamm, Greenwald, Bradley, & Lang, 1993) as well as higher FPSR (Hamm et al., 1993; Lipp, Sheridan, & Siddle, 1994; Lissek et al., 2008) to CS+ than CS- have also been demonstrated in healthy humans.

Neuroimaging studies show activations in the amygdala (Phelps et al., 2001; Phelps, Delgado, Nearing, & Ledoux, 2004; Milad et al., 2007b; Hermans et al., 2016), dorsal anterior cingulate cortex (dACC; Milad et al., 2007a; Hermans et al., 2016; Phelps et al., 2004), hippocampus (Hermans et al., 2016), and insular cortex (Hermans et al., 2016) and deactivation in the ventromedial prefrontal cortex (vmPFC; Milad et al., 2007b) during fear conditioning to CS+ versus CS- in healthy adults. The dACC in human is homologue to the prelimbic cortex in rats and both project to the lateral nucleus of the amygdala. The vmPFC in human is homologue to the infralimbic cortex and both project to the basal nucleus and to the intercalated cells of the amygdala.

In 2004, Phelps and colleagues showed that there was increased activation in the amygdala, dACC, and subgenual anterior cingulate region to CS+ versus CS- in healthy adults.

Additionally, they observed greater blood-oxygen-level-dependent (BOLD) response in the vmPFC to CS- versus CS+. Milad and colleagues (2007b) observed deactivations in the vmPFC to CS+ versus CS-. Replicating the work of Phelps and colleagues (2004), Hermans and colleagues (2016) observed differential BOLD responses to CS+>CS- during acquisition in the amygdala and hippocampus as well as the dACC, anterior insula, and temporoparietal junction in healthy adults (Hermans et al., 2016).

Similarly, Milad and colleagues (2007a) reported dACC activation to CS+ relative to the CS- during conditioning. They also found positive correlation between the cortical thickness within the dACC and conditioned fear responses to the SCR of CS+ relative to the CS- during conditioning in healthy adults. In 2007, Milad and colleagues (2007b) found significant right dorsal amygdala activation and deactivation of the vmPFC to the CS+ relative to the CS- during early fear conditioning in healthy adults.

Psychophysiology and Neurocircuitry of Fear Conditioning in PTSD

PTSD has been associated with heightened psychophysiological responses during fear conditioning. Greater SCR, HRR, and FPSR have been observed in PTSD relative to non-PTSD in response to CS+ versus CS-, indicative of heightened fear conditioning (Orr et al., 2000; Wessa and Flor, 2007; Blechert et al., 2007; Jovanovic et al., 2010; Glover et al., 2011). However, this finding is not consistent throughout the literature. Peri and colleagues (2000) found larger responses to CS- during acquisition in the PTSD versus trauma-unexposed control group. Grillon & Morgan (1999) found opposite results of greater startle response to CS+ compared to CS- in combat-exposed *non*-PTSD veterans than in veterans with PTSD. Some studies have found no differences in the two groups during conditioning (Orr, Lasko, Shalev, & Pitman, 1995; Orr, Lasko, Metzger, & Pitman, 1997; Bremner et al., 2005; Blechert et al., 2007;

Milad et al., 2008; Milad et al., 2008; Milad et al., 2009; Glover et al., 2011; Garfinkel et al., 2014; Zuj et al., 2016). Although the literature is mixed, there is some evidence for less discrimination between threat and safety cues in PTSD (Grillon & Morgan, 1999).

Varying activations in the amygdala (Bremner et al., 2005; Garfinkel et al., 2014) and prefrontal cortex (Rougemont-Bucking et al., 2011; Garfinkel et al., 2014) have been observed in individuals with PTSD compared to trauma-exposed non-PTSD and healthy individuals during conditioning. Compared to women without abuse PTSD, women with early childhood sexual-abuse-related PTSD showed greater left amygdala activity during fear conditioning to CS+ (Bremner et al., 2005). Rougemont-Bucking and colleagues (2011) reported significantly greater dACC activation in those with PTSD than trauma-exposed non-PTSD controls during context presentation during late conditioning. However, Garfinkel and colleagues (2014) did not find any difference between PTSD patients and combat-exposed non-PTSD participants. Both groups showed increased BOLD signal to CS+ compared with CS- in key areas implicated in fear learning including amygdala, brainstem, insula, and ACC.

Current Study

Although previous research has shown abnormal SCR and brain activation during fear conditioning in PTSD, the origin of these abnormalities remain unclear. These abnormalities may reflect an acquired characteristic of PTSD, the effect of trauma exposure itself independent of the development of PTSD, or a vulnerability factor for developing PTSD after trauma exposure (Pitman et al., 2006). It is important to determine the origin of the abnormalities associated with fear conditioning in PTSD because the origin has potential clinical implications for primary prevention and/or early treatment of PTSD.

If an abnormality is an acquired characteristic of PTSD, it can help in the diagnosis of PTSD or in the assessment of treatment response. If an abnormality is a vulnerability factor for PTSD (may increase the risk of PTSD after trauma exposure or result from trauma exposure), it can be identified before potential exposure to traumatic events and help in primary or secondary prevention efforts. Diminished vmPFC activation during the recollection of stressful versus neutral events was found to be an acquired characteristic of PTSD (Dahlgren et al., in press) and can be used to inform diagnosis and the assessment of treatment response. Increased amygdala (Admon et al., 2009; Shin et al., 2009) and dACC activation during cognitive interference (Shin et al., 2011) have been identified as vulnerability factors in PTSD and can aid in primary or secondary prevention for PTSD. Specifically, Admon and colleagues (2009) showed that high levels of amygdala reactivity before a stressful event was related to greater increase in stress symptoms later in time.

Methods

Design

To understand the origins of these abnormalities in PTSD, we used a case-control design including male monozygotic twin pairs discordant for combat (see Figure 1). Participants were trauma-exposed individuals with PTSD (ExP+) and their trauma-unexposed identical co-twins without PTSD (UxP+) as well as trauma-exposed individuals without PTSD (ExP-) and their trauma-unexposed co-twins without PTSD (UxP-).

This design allows for an understanding of origin in the following ways: Abnormalities found in both members of the P+ pairs compared to the P- pairs would indicate a familial vulnerability. Abnormalities found in the exposed participants (ExP+ and ExP-) compared to unexposed participants (UxP+ and UxP-) would be viewed as an effect of combat exposure.

Abnormalities found only in ExP+ compared to the other three groups should reflect an acquired characteristic of PTSD.

Twin Study Design

Analyses of this twin design were conducted using a 2 (PTSD Diagnosis: P+ pairs, P- pairs) x 2 (Combat Exposure: Ex participants, Ux participants) mixed-model analysis of variance (ANOVA). PTSD Diagnosis is a between subjects factor and Combat Exposure is a within-subjects factor. The following effects may be observed:

Main effect of diagnosis: familial vulnerability factor. If there is a statistically significant difference between the two PTSD Diagnosis groups (P+ vs. P-) and there is no interaction between PTSD Diagnosis and Combat Exposure, the biological abnormality in PTSD may be attributed to familial vulnerability factors.

Main effect of exposure: trauma exposure. If there is a statistically significant difference between the Combat Exposure groups (Ex versus Ux) on the dependent variable and no interaction between PTSD Diagnosis and Combat Exposure, the biological abnormality may be attributed to exposure to combat, regardless of PTSD.

Diagnosis * exposure interaction: acquired characteristic of PTSD. If the interaction between PTSD Diagnosis group and Combat Exposure is significant, and the combat veterans with PTSD (ExP+) show the abnormality but all three other groups do not, then this would imply that the abnormalities are acquired characteristics of PTSD.

Participants

Participants were recruited from the Vietnam Era Twin Registry (VETR), University of Washington Twin Registry (UWTR), and an online advertisement. IRB approval for this project

was received through the Partners Healthcare IRB at Massachusetts General Hospital (MGH) and the Department of Veterans Affairs.

SCR data were available for 25 twin pairs (50 individuals) from the fear conditioning phase of the paradigm. Of the 25 twin pairs, 11 were P+ twin pairs and 14 were P- twin pairs. Neuroimaging data were available for 29 twin pairs (58 individuals). Of the 29 twin pairs, 12 were P+ twin pairs and 17 were P- twin pairs.

Demographics and Psychometrics

After providing written informed consent, each participant was administered the Clinician-Administered PTSD Scale (CAPS; Weathers et al., 2001) to assess PTSD diagnosis, the Structured Clinical Interview for DSM-IV (SCID; First et al., 2002) to determine the presence of other mental disorders, and a number of different psychometric assessments to assess mood, personality, and behavior (see Table 1 and 2).

Procedures

Task Procedures. For the current study, the fear conditioning and extinction paradigm described by Milad and colleagues (2009) was used. The paradigm includes five phases that were run over the course of two days: habituation, fear conditioning and extinction learning (run on day 1), and extinction recall and renewal (run on day 2). We will be focusing only on the conditioning phase in this paper.

The five different phases consisted of two different visual contexts (CXs), an office and library, with an unlit lamp in each room. Photographs of these two contexts were displayed on a computer monitor to participants in a magnetic resonance imaging scanner. The CSs in the paradigm were different colors of the lit lampshades (e.g., red, blue, yellow) that were counterbalanced throughout the experiment. The CS+s (e.g., blue and red lamp colors) predicted

the US (a finger shock that occurs 62.5% of the time) whereas the CS-s (e.g., a yellow colored lamp) never predicted shock.

On day 1 during habituation, images of the two different rooms with a lamp in it were pre-exposed to participants. The habituation phase consisted of eight trials with the two types of to-be CS+ and to-be CS- (four of each) within either the to-be conditioning context (CX+) or the to-be extinction context (CX-). The CS+ and CS- were counterbalanced across subjects (i.e., different color lights and contexts). During the conditioning phase, two different colored lights (CS+1 and CS+2) predicted a mild electric shock (US). The conditioning phase consisted of 8 CS+1, 8 CS+2, and 16 CS- trials. At the beginning of the phase, a blank screen appeared for 12, 15, or 18 seconds and was immediately followed by an image of the context with the unlit lamp light. After 3 seconds, the cue or lit lamp appeared and a shock was delivered immediately after 6 seconds (at the offset of the CS presentation). Of the 16 CS+ trials, there were 5 shock trials and 3 omitted shock trials for CS+1 and 5 shock trials and 3 omitted shock trials for CS+2.

SCR Procedures. Prior to running the task, electrodes were placed on the hypothenar surface of the non-dominant hand to measure skin conductance. Electrodes were also attached to the fingers of the participant's right hand to allow for delivery of electric stimulation (via a Coulbourn Transcutaneous Aversive Finger Stimulator). Prior to starting the experiment, participants were asked to determine the intensity of the stimulation that would be highly annoying but not painful. SCR was measured throughout the different phases of the experiment. There were no group differences in the shock levels (milliampere; mA) chosen by the participants and in the skin conductance levels (uSiemens; uS) at the beginning of the experiment (see Table 1).

Functional MRI Procedures. A Trio 3.0 Tesla whole body high-speed imaging device (Siemens Medical Systems, Iselin, New Jersey) with a 12-channel gradient head coil was used. As previously described by our group in Milad et al. (2009), an automated scout image was done to obtain initial positioning of the brain. This was followed by shimming procedures and a high-resolution 3D magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence (TR=2.530 ms, TE1=1.64 mm, TE2=3.5 mm, TE3=5.36 ms, TE4=7.22 ms, flip angle=7 °, 1x1mm in plane x 1.0 mm) for spatial normalization and for positioning of subsequent scans. Functional MRI images (i.e., blood oxygenation level dependent [BOLD]) were acquired with gradient echo T2*-weighted sequence (TR/TE/Flip angle = 2.560 ms/30 ms/90°) in forty-eight 2.5 mm axial slices (0.5 mm skip). The same scanning procedure was conducted on Day 2.

Data Analysis

SCR Data Analysis. Psychophysiological data were completed using IBM SPSS Statistics (version 22). SCR for CS+ trials versus CS- trials and early CS+ versus early CS- were calculated during conditioning and will be referred to as differential SCR for overall conditioning and differential SCR for early conditioning, respectively, throughout this paper. To calculate SCR for each CS trial, the mean skin conductance level during the 2 seconds immediately prior to the CS onset when an image of the context with the unlit lamplight appeared was subtracted from the highest skin conductance level recorded during the 6 seconds CS duration. Each SCR was square-root transformed prior to analysis (Milad et al., 2008). Differential SCR for overall conditioning was calculated by subtracting skin conductance levels for the average of all trials of CS- from the average of all trials of CS+. Differential SCR for early conditioning was calculated by subtracting skin conductance levels for the average of the first 4 trials of CS- from the average of the first 4 trials of CS+. SCR for the UCR to shock was

also assessed. Additional correlational analyses were performed on SCR data with PTSD symptom severity, as measured by total CAPS score.

Functional MRI Data Analysis. Functional MRI data were analyzed with the Statistical Parametric Mapping (SPM) version 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8), which is run in MATLAB (MathWorks). All data was preprocessed including slice timing, realignment to correct for motion, coregistration of an individual's T1 image to their EPI image, normalization to wrap an individual's brain into standard space based on the MNI template, and smoothing. Participants with translation greater than or equal to 6 mm and rotation greater than or equal to 4º were excluded. To estimate the stimulus effects at each voxel, an event-related design was used. Statistical parametric maps were calculated according to a general linear model for the contrasts of interest across time. Contrasts for the differences between CS+ versus CS- and early CS+ versus CS- were assessed during conditioning. Two types of analyses were used: those conducted on (1) whole-brain voxelwise functional data and (2) functional data extracted from functional regions of interest (ROIs), including the amygdala, dACC, hippocampus, and vmPFC, that emerged from the voxelwise analyses. These areas were defined by functional activations in the voxelwise maps with z-scores greater than 3.09. The ROIs were centered around the maximum voxel and had a sphere radius of 4 mm. The statistical parametric maps resulting from voxelwise analyses were inspected and analyzed for the main effect of PTSD Diagnosis, main effect of Combat Exposure, and the PTSD Diagnosis * Combat Exposure interaction. Additional correlational analyses were performed on the data extracted from functional ROIs with CAPS, with differential SCR for early conditioning, and with SCR for UCR to shock.

Hypotheses

SCR. We expect to observe greater differential SCR for early conditioning in the ExP+ versus ExP- groups. A directional hypothesis regarding the origin of greater differential SCR for early conditioning in the twins cannot be made based on previous research.

Functional MRI. We expect to observe greater activations in the amygdala, dACC, hippocampus, and insula and deactivation in the vmPFC during early conditioning in the ExP+ group relative to the ExP- group. A directional hypothesis regarding the origin of activations in these areas in the twins cannot be made based on previous research.

Results

We will focus our analyses on early conditioning (the first 4 trials of each CS+ versus the first 4 trials of CS-) for SCR and functional brain activation as these trials reflect immediate changes due to conditioning. We also assessed differential SCR for overall conditioning (16 trials of CS+ versus 16 trials of CS-) and found similar results. Thus, we will focus on only the early conditioning for SCR and functional brain activation in this paper.

Psychophysiological Responses During Fear Conditioning

We assessed differential SCR for early conditioning in the following analyses.

ExP+ versus ExP-. The ExP+ twins had lower differential SCR for early conditioning than the ExP- twins, $t(23)=2.19$, $p=.039$.

Main Effect of Diagnosis. An ANOVA revealed a significant main effect of PTSD Diagnosis, $F(1,23)=4.77$, $p=.039$. This shows that individuals with PTSD and their co-twins have difficulty learning to distinguish between the threat (CS+) and safety cues (CS-) during early conditioning, and this may be a PTSD familial vulnerability factor (see Figure 2 and Figure 3).

Main Effect of Exposure. There was no significant main effect of Trauma Exposure, $F(1,23)=1.49$, $p=.23$.

Diagnosis * Exposure Interaction. There was no significant interaction effect, $F(1,23)=1.85$, $p=.19$.

SCR for UCR to Shock. We assessed SCR for the UCR to shock and found no significant difference between ExP+ and ExP- twins ($t(23)=-0.943$, $p=.355$), and no main effect of Diagnosis ($F(1,23)=0.473$, $p=.499$), main effect of Exposure ($F(1,23)=0.014$, $p=.906$), or Diagnosis * Exposure interaction ($F(1,23)=0.537$, $p=.471$.

SCR and CAPS Correlations

Correlation of combat-exposed twins' SCR with their own CAPS. Differential SCR for early conditioning in the combat-exposed twins were negatively correlated with their own total CAPS scores, $r(23)=-0.508$, $p=.010$ (see Figure 4).

Correlation of combat-unexposed twins' SCR with their combat-exposed co-twins' CAPS. Differential SCR for early conditioning in the combat-unexposed twins were not significantly correlated with their combat-exposed co-twins' total CAPS score, $r(23)=-0.353$, $p=.084$, although a trend was present (see Figure 5).

Functional Activations During Early Fear Conditioning

We used the contrast of Early CS+ versus Early CS- (i.e., early conditioning) in the following fMRI analyses.

ExP+ versus ExP-. The ExP+ twins showed greater activation in the left insula (MNI: x=-32, y=2, z=12) and right insula (MNI: x=46, y=-6, z=8) and less rACC (MNI: x=14, y=42, z=6) activation than the ExP- twins (see Table 3 and Figures 6-8).

Main Effect of Diagnosis. The PTSD twins and their co-twins showed greater activation in the left insula (MNI: x=-34, y=0, z=10; MNI: x=-34, y=-22, z=20) and right insula (x=36, y=-24, z=8) than the non-PTSD twins and their co-twins, which may reflect a PTSD familial vulnerability factor (see Table 3 and Figures 9-11).

Main Effect of Exposure. The exposed twins showed less rACC activation than the unexposed twins, which may be attributed to exposure to combat (see Table 3 and Figure 12).

Diagnosis * Exposure Interaction. There was a significant interaction between PTSD Diagnosis and Combat Exposure in the dACC (see Table 3 and Figure 13). However, this interaction does not reflect a clear difference between ExP+ and all other groups. The ExP+ twins and the UxP- twins showed less dACC activation than the UxP+ twins and the ExP- twins. This pattern is difficult to interpret.

Functional Brain Activation and CAPS Correlation

ExP+ versus ExP-.

Correlation of ExP+ twins' brain activation with their own CAPS. BOLD signal changes in the left insula (-32, 2, 12), the right insula (46, -6, 8), and rACC (14, 42, 6) during early conditioning in the combat-exposed twins with PTSD were not correlated with their own total CAPS score, r(10)=-0.44, p=.15, r(10)=0.31, p=.32, and r(10)=0.097, p=.77, respectively.

Main Effect of Diagnosis.

Correlation of combat-exposed twins' brain activation with their own CAPS. BOLD signal changes in the right insula (36, -24, 8) and left insula (-34, -22, 20) during early conditioning in the combat-exposed twins were not significantly correlated with their own total CAPS score, r(27)=0.273, p=.152 and r(27)=0.163, p=.399, respectively. BOLD signal changes

in the left insula (-34, 0, 10) during early conditioning in the combat-exposed twins were positively correlated with their own total CAPS score, r(27)=0.439, p=.017 (see Figure 14).

Correlation of combat-unexposed twins' brain activation their combat-exposed co-twins' CAPS. BOLD signal changes in the right insula (36, -24, 8) and left insula (-34, 22, 20) during early conditioning in the combat-unexposed twins were positively correlated with their combat-exposed co-twins' total CAPS score, r(27)=0.452, p=.014 (see Figure 15) and r(27)=0.524, p=.004 (see Figure 16), respectively. BOLD signal changes in the left insula (-34, 0, 10) during early conditioning in the combat-unexposed twins were not significantly correlated with their combat-exposed co-twins' total CAPS score, r(27)=0.360, p=.055 (see Figure 17), although a trend was present.

Correlation Between Functional Brain Activation and Differential SCR for Early Conditioning

ExP+versusExP-.

Correlation of ExP+ twins' brain activation with their own SCR. BOLD signal changes in the left insula (-32, 2, 12), right insula (46, -6, 8), and rACC (14, 42, 6) during early conditioning in the combat-exposed twins with PTSD were not correlated with their own differential SCR for early conditioning, r(9)=0.079, p=.818, r(9)=-0.117, p=.731, and r(9)=0.270, p=.422, respectively.

Correlation of ExP- twins' brain activation with their own SCR. BOLD signal changes in the left insula (-32, 2, 12), right insula (46, -6, 8), and rACC (14, 42, 6) during early conditioning in the combat-exposed twins without PTSD were not correlated with their own differential SCR for early conditioning, r(12)=0.366, p=.198, r(12)=0.054, p=.853, and r(12)=-0.115, p=.696, respectively.

Main Effect of Diagnosis.

Correlation of combat-exposed twins' brain activation with their own SCR. BOLD signal changes in the left insula (-34, 0, 10), left insula (-34, -22, 20), and right insula (36, -24, 8) during early conditioning in the combat-exposed twins were not significantly correlated with their own differential SCR for early conditioning, r(23)=-0.090, p=.670, r(23)=-0.106, p=.612, and r(23)=-0.323, p=.115, respectively.

Correlation of combat-unexposed twins' brain activation with their own SCR. BOLD signal changes in the left insula (-34, 0, 10), left insula (-34, -22, 20), and right insula (36, -24, 8) during early conditioning in the combat-unexposed twins were not significantly correlated with their own differential SCR for early conditioning, r(23)=-0.018, p=.933, r(23)=-0.129, p=.539, and r(23)=-0.174, p=.406, respectively.

Correlation Between Functional Brain Activation for Early Conditioning and SCR for UCR to Shock

ExP+versusExP-.

Correlation of ExP+ twins' brain activation with their own SCR. BOLD signal changes in the left insula (-32, 2, 12), right insula (46, -6, 8), and rACC (14, 42, 6) during early conditioning in the combat-exposed twins with PTSD were not correlated with their own SCR for the UCR to shock, r(9)=-0.161, p=.635, r(9)=0.415, p=.204, and r(9)=-0.103, p=.764, respectively.

Correlation of ExP- twins' brain activation with their own SCR. BOLD signal changes in the left insula (-32, 2, 12), right insula (46, -6, 8), and rACC (14, 42, 6) during early conditioning in the combat-unexposed twins without PTSD were not correlated with their own

SCR for the UCR to shock, r(12)=-0.378, p=.183, r(12)=-0.113, p=.699, and r(12)=-0.398, p=.165, respectively.

Main Effect of Diagnosis.

Correlation of combat-exposed twins' brain activation with their own SCR. BOLD signal changes in the left insula (-34, 0, 10) and left insula (-34, -22, 20) during early conditioning in the combat-exposed twins were negatively correlated with their own SCR for the UCR to shock, r(23)=-0.402, p=.046 (see Figure 18) and r(23)=-0.534, p=.006 (see Figure 19), respectively. BOLD signal changes in the right insula (36, -24, 8) during early conditioning in the combat-exposed twins were not correlated with their own SCR for the UCR to shock, r(23)=-0.014, p=.946. Increased left insula activation was related to decreased UCR to shock in the combat-exposed twins.

Correlation of combat-unexposed twins' brain activation with their own SCR. BOLD signal changes in the left insula (-34, 0, 10), left insula (-34, -22, 20), and right insula (36, -24, 8) during early conditioning in the combat-unexposed twins were not correlated with their own SCR for the UCR to shock, r(23)=-0.209, p=.233, r(23)=0.258, p=.235, and r(23)=-0.014, p=.946 respectively.

Discussion

The purpose of this study was to determine whether the origins of the psychophysiological and neural abnormalities observed in fear conditioning in PTSD are attributable to familial vulnerability factors, effects of trauma exposure, or acquired characteristics of PTSD.

Decreased differential SCR was observed in the ExP+ twins relative to the ExP- twins during early conditioning. Additionally, combat veterans with PTSD and their identical co-twins

showed lower differential SCR to early conditioning relative to combat veterans without PTSD and their identical co-twins. These results suggest that decreased ability to distinguish between threat and safety cues is a familial risk factor for PTSD. Furthermore, total CAPS scores in the combat-exposed twins (1) were significantly negatively correlated with their differential SCR for early conditioning and (2) tended to negatively correlate with their combat-unexposed co-twins' differential SCR for early conditioning. This significant relationship further supports the claim that decreased differential SCR in PTSD is a vulnerability factor because the unexposed co-twins' SCR predicts their combat-exposed co-twins' PTSD symptom severity.

The SCR results are not in line with previous findings of increased differential SCR in PTSD during conditioning (Orr et al., 2000; Wessa and Flor, 2007; Blechert et al., 2007; Jovanovic et al., 2010; Glover et al., 2011). However, they are similar to the findings of Grillon and Morgan (1999) who found less of a startle response to CS+ relative to CS- in combat-exposed PTSD veterans than in combat-exposed non-PTSD veterans. Our results are suggestive of decreased differential conditioning in PTSD or greater differential conditioning in non-PTSD. The differences observed between PTSD and non-PTSD in our study are also not attributable to the shock level chosen by the participant or the UCS to shock. However, these differences may be attributable to time since trauma. Specifically, the twins who participated in this study had experienced combat-exposure over 42-63 years ago in the Vietnam war and have chronic PTSD. This gap between time since trauma and assessment of SCR to fear conditioning may lead to variability in the SCR seen in our study and previous literature.

In addition to decreased SCR in PTSD, greater left insula and right insula activation and less rACC activation were observed in the ExP+ group relative to the ExP- group for early conditioning. Greater activation in two regions of the left insula and right insula was also

observed in the PTSD twins and their co-twins than the non-PTSD twins and their co-twins. Moreover, CAPS scores in the combat-exposed twins were positively correlated with both their own and their unexposed co-twins' activation in the left and right insula. These results suggest that insula hyperresponsivity is a familial vulnerability factor for PTSD. Increased insula activation in PTSD is consistent with previous literature and our hypothesis. Additionally, although increased insula activation was not associated with differential SCR to early conditioning, it was associated with UCR to shock. Thus, increased insula may be related to the awareness of the shock given its role in monitoring bodily states such as anxiety and pain (VanElzakker et al., 2014).

Furthermore, decreased rACC activation was observed in the exposed twins than the unexposed twins. These results suggest that decreased rACC activation is attributable to combat exposure. Lastly, an interaction was observed between a PTSD diagnosis and combat exposure in the dACC. Although these effects were observed, the patterns in these regions were unclear and difficult to interpret, as a difference was not seen between the combat-exposed twins with PTSD from the other three groups.

Overall, decreased SCR and increased left and right insula activations are vulnerability factors for PTSD. These abnormalities can be used in primary and/or secondary prevention efforts. Primary prevention would involve screening for decreased SCR and/or increased left and right insula activation prior to trauma exposure and may be used to screen for career choices or for situations/settings to avoid. Secondary prevention would involve screening for these vulnerability factors after trauma exposure to assess for the increased risk of developing PTSD. Secondary prevention can occur in situations where an individual may be exposed to unexpected

trauma such as a natural disaster and may be screened post-trauma for preventive measures to decrease the risk of PTSD.

Although screening for such vulnerability factors for the prevention of PTSD may be beneficial, screening can also raise ethical concerns for such individuals. Specifically, individuals who may be interested in careers with increased risk for exposure to trauma such as the army, firefighters, police officers, and pilots, may find themselves screened out of these fields. Future research also needs to be conducted to assess the cutoff criterion needed to screen individuals for prevention. Although it is important to set guidelines and criterion for screening, this can also lead to increased false alarms and misses. Thus, it is important to fully understand both the positive and negative roles of these vulnerability factors for PTSD and the role they play in prevention and other applications.

Limitations and Future Directions

The finding of decreased SCR in PTSD is not consistent with the previous literature and our hypothesis of increased SCR in PTSD. The differences seen in the fear conditioning data in this study as compared to other studies may also be attributable to the various different forms of fear conditioning and psychophysiological measures used in the literature. Some studies used colored lights with electric shock (Grillon & Morgan, 1999; Milad et al., 2008; Milad et al., 2009; and Garfinkel et al., 2014) as their CS and UCS whereas some studies used various colored shapes or visual cues with electric shock or airblast (Peri et al., 2000; Orr et al., 2000; Bremner et al., 2005; Wessa and Flor, 2007; Blechert et al., 2007; Jovanovic et al., 2010; Glover et al., 2011). Furthermore, varying forms of psychophysiological measures such as SCR, FPSR, and HRR responses have been used and show varying results. Glover et al. (2011) used both SCR and FPSR however they only found heightened fear in PTSD with FPSR but not SCR. A

number of studies have also shown that SCR may not be as sensitive of a measure as FPSR for assessing differences in fear expression (Jovanovic et al., 2010; Glover et al., 2011; Wessa and Flor, 2007). Most importantly, this study consisted of a small sample size of elderly, Caucasian males who were exposed to trauma over 40 years ago. This further limits us in understanding and comparing our results to the findings of our studies.

In future analyses, it will be important to analyze and interpret the findings of SCR and functional brain activation in the extinction, recall, and renewal phase to get a thorough understanding of fear conditioning and extinction in PTSD.

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Table 1

Demographic and Clinical Characteristics for Physiological SCR Data of Combat-Exposed Vietnam Veterans With and Without PTSD and Their Unexposed, Identical Co-twins

	Mean (SD)				Mixed Model ANOVA					
	PTSD Pair		Non-PTSD Pairs		Diagnosis		Exposure		Interaction	
	Exposed (n=11)	Unexposed (n=11)	Exposed (n=14)	Unexposed (n=14)	F	P Value	F	P Value	F	P Value
Age	61.64 (6.98)		62.79 (4.00)		.27*	.61				
Education	14.09 (2.91)	13.64 (3.96)	15.82 (3.64)	14.93 (2.79)	1.41*	.25	2.28*	.15	.24*	.63
CAPS score	51.91 (25.61)	2.55 (3.21)	2.50 (3.94)	1.79 (4.00)	48.72*	<.001	51.62*	<.001	48.72*	<.001
BDI score	8.00 (7.20)	2.18 (1.99)	4.00 (3.98)	3.57 (3.44)	4.86*	.038	6.53*	.02	4.86*	.04
BAI score	10.00 (10.94)	1.73 (2.61)	2.57 (1.79)	3.71 (5.50)	2.46*	.131	4.27*	.05	7.44*	.01
Shock level (mA)	2.31 (0.91)	2.31 (0.90)	2.21 (0.60)	1.96 (0.37)	1.01*	.326	0.54*	.47	0.54*	.47
Skin conductance level (uS)	3.39 (1.83)	4.45 (2.68)	3.10 (1.77)	2.88 (2.19)	1.59†	.221	0.78†	.386	1.81†	.192

* = df(1,23), † = df(1,22)

Table 2

Demographic and Clinical Characteristics for Functional Brain Activation Data of Combat-Exposed Vietnam Veterans With and Without PTSD and Their Unexposed, Identical Co-twins

	Mean (SD)				**Mixed Model ANOVA**					
	PTSD Pair		**Non-PTSD Pairs**		**Diagnosis**		**Exposure**		**Interaction**	
	Exposed (n=12)	**Unexposed (n=12)**	**Exposed (n=17)**	**Unexposed (n=17)**	**$F_{1,27}$**	**P Value**	**$F_{1,27}$**	**P Value**	**$F_{1,27}$**	**P Value**
Age	61.75 (6.66)		62.53 (3.68)		.16	.69				
Education	14.00 (2.80)	13.83 (3.83)	15.38 (3.52)	14.59 (2.65)	.88	.36	1.36	.25	.58	.45
CAPS score	55.17 (26.90)	2.33 (3.14)	5.47 (8.92)	2.06 (4.19)	50.21	<.001	61.35	<.001	47.37	<.001
BDI score	10.17 (10.17)	2.00 (2.00)	4.71 (3.97)	4.24 (3.80)	1.18	.29	8.56	.007	6.79	.02
BAI score	9.92 (10.43)	1.58 (2.54)	2.47 (1.74)	5.06 (6.99)	1.48	.24	2.89	.10	10.46	.003

Table 3

Voxelwise Analysis Results for Early Conditioning

	Region	z Score	MNI Coordinates				
			x	y	z	p_{uncorr}	Cluster Size (k_E)
ExP+ versus ExP-							
PTSD > non-PTSD	Left Insula	3.66	-32	2	12	<.001	18
	Right Insula	3.42	46	-6	8	<.001	27
Non-PTSD > PTSD	rACC	3.11	14	42	6	<.001	1
Main Effect of Diagnosis							
PTSD pairs > non-PTSD pairs	Right Insula	4.21	36	-24	8	<.001	121
	Left Insula	3.24	-34	0	10	<.001	7
	Left Insula	3.89	-34	-22	20	<.001	79
Main Effect of Exposure							
Unexposed > Exposed	rACC	3.15	16	46	4	<.001	2
Interaction							
Non-PTSD > PTSD	dACC	3.20	-6	14	32	<.001	1

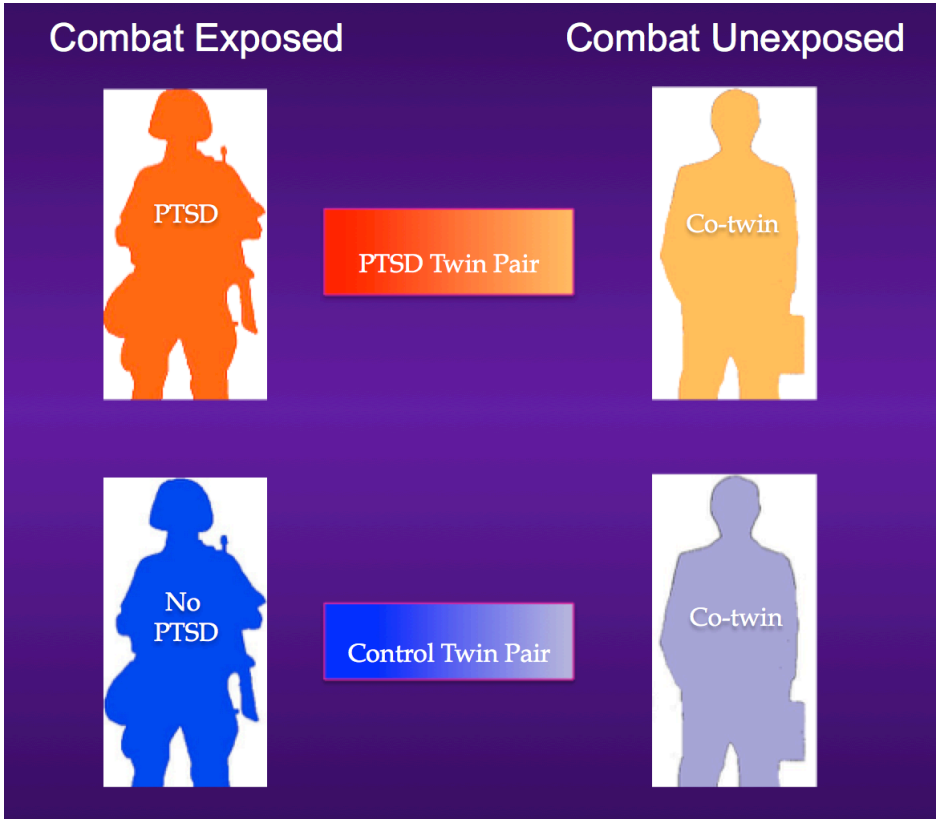

Figure 1. Case-control design including male monozygotic twin pairs discordant for combat.

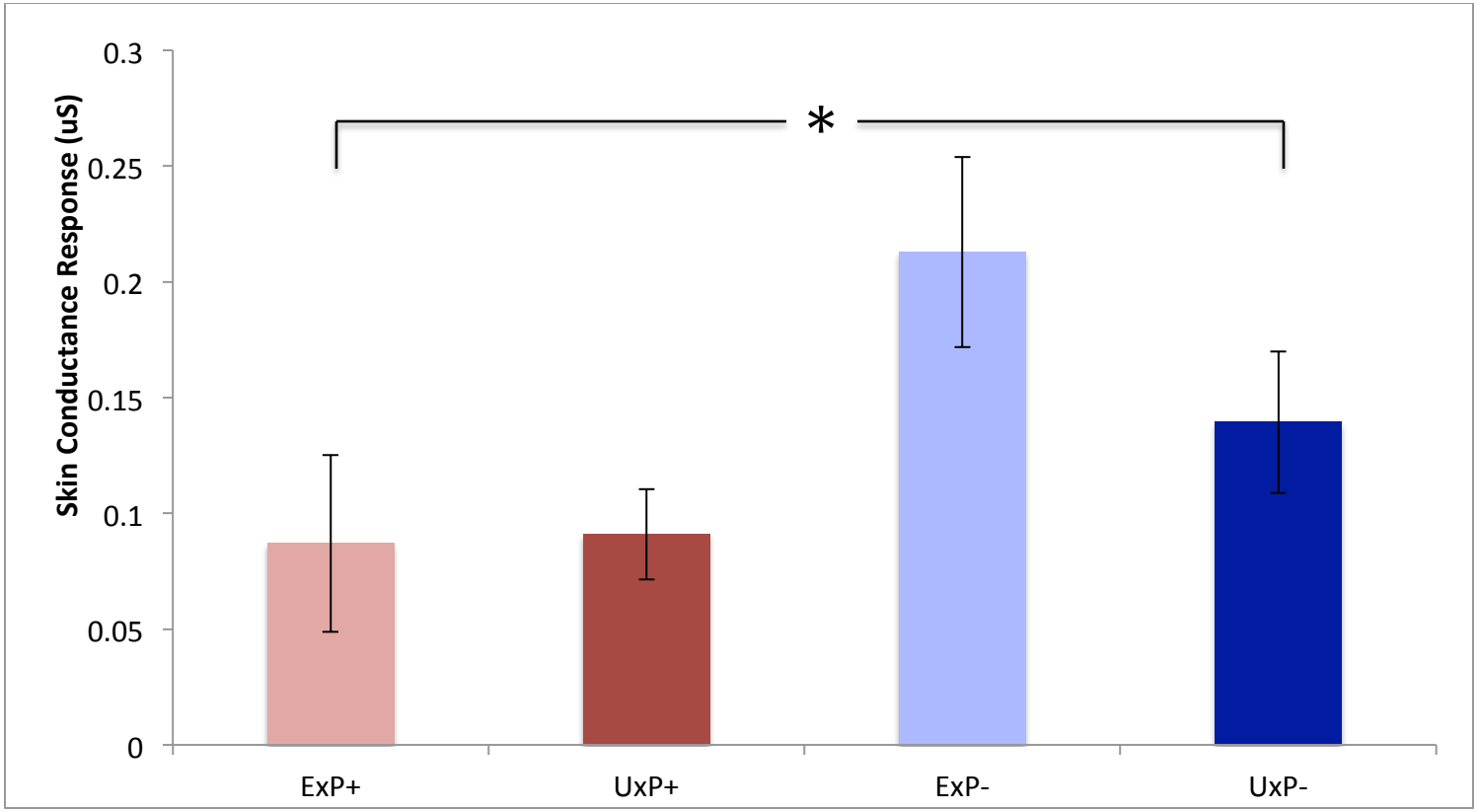

Figure 2. Differential SCR for early conditioning. Skin conductance responses (SCRs) to the early conditioned stimulus (CS) trials that were paired with shock (CS+) minus the early CS trials that was never paired with shock (CS-) in the PTSD exposed group (ExP+), PTSD unexposed group, (UxP+), non-PTSD exposed group (ExP-), and non-PTSD unexposed group (UxP-).

* = Main effect of PTSD Diagnosis, $F(1,23)=4.77$, $p=.039$.

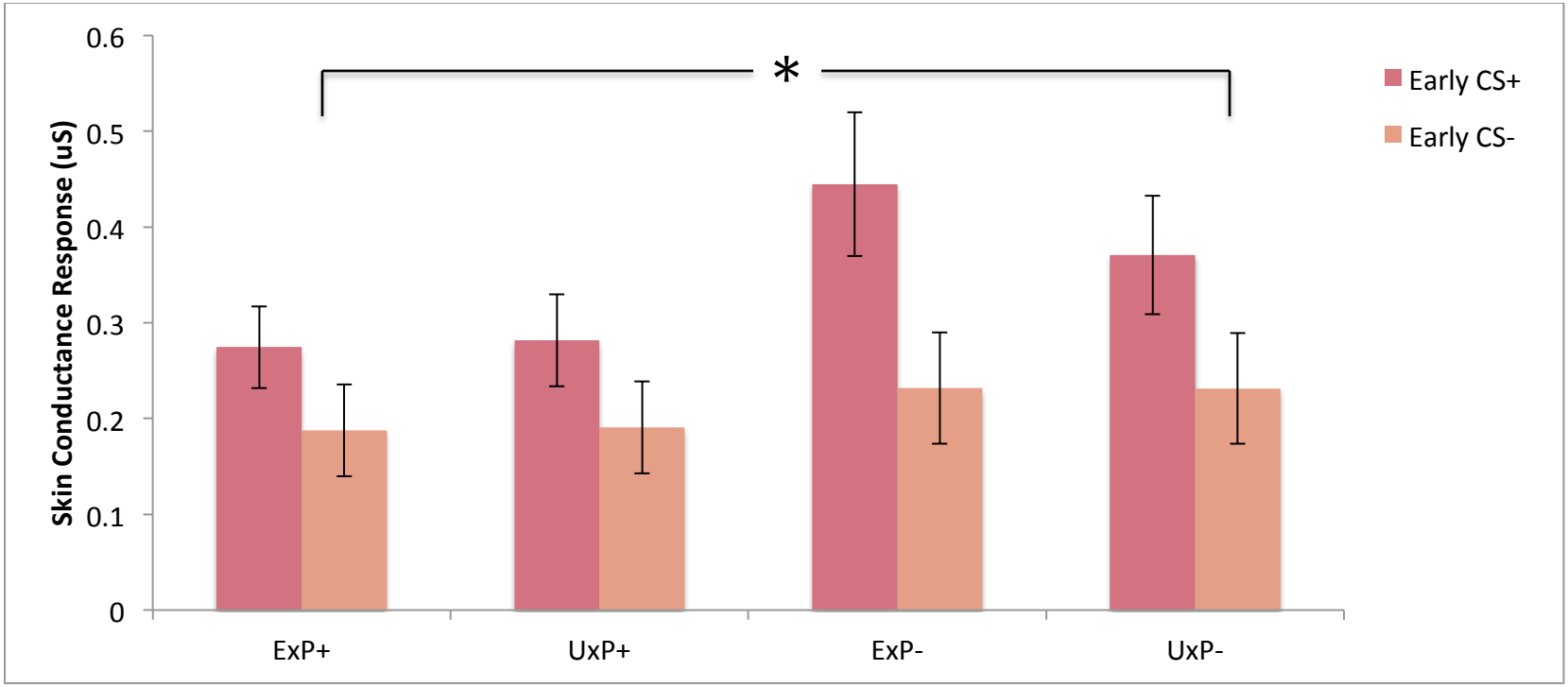

Figure 3. SCR for early CS+ trials and early CS- trials. SCRs to the first 4 conditioned stimulus (CS) trials that were paired with shock (CS+) minus the first 4 CS trials that were never paired with shock (CS-) in the PTSD exposed group (ExP+), PTSD unexposed group (UxP+), non-PTSD exposed group (ExP-), and non-PTSD unexposed group (UxP-).

*= Stimulus * PTSD Diagnosis interaction, $F(1,23)=4.77$, $p=.039$ (see Appendix A).

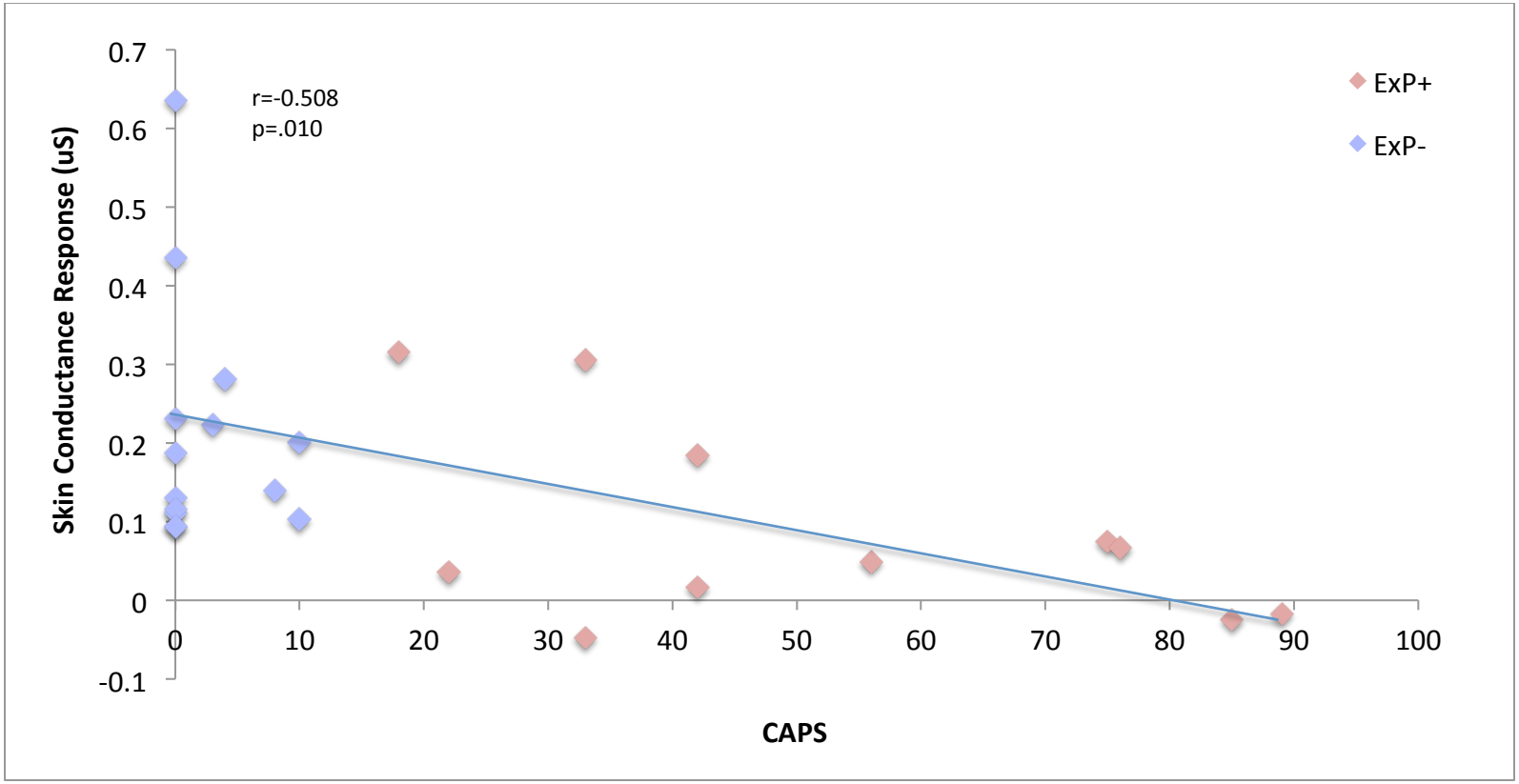

Figure 4. Correlation of combat-exposed twins' differential SCR for early conditioning with their own CAPS.

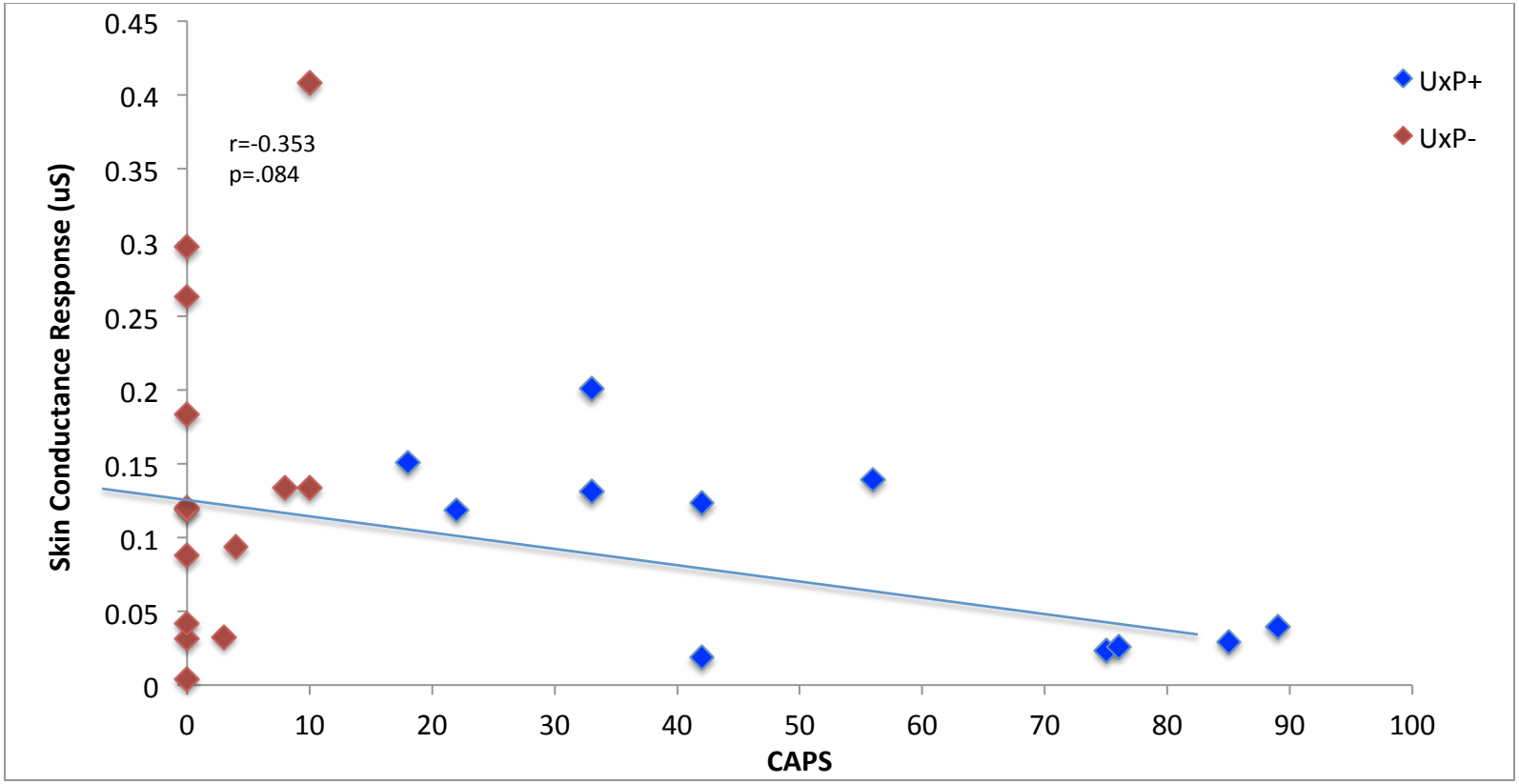

Figure 5. Correlation of combat-unexposed twins' differential SCR for early conditioning with their combat-exposed co-twins' CAPS.

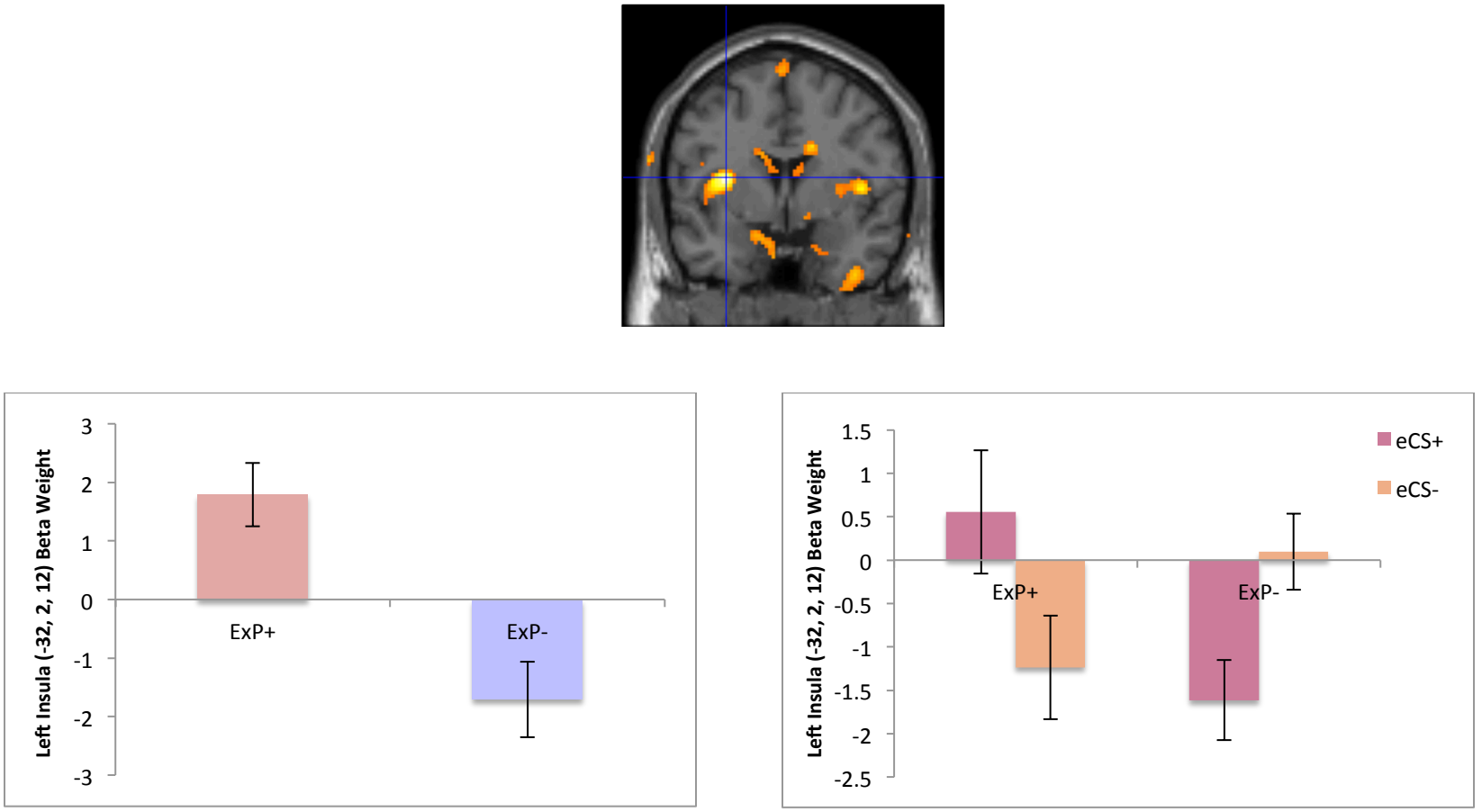

Figure 6. Left insula (MNI: x=-32, y=2, z=12) activation in combat-exposed twins with PTSD (ExP+) and combat-exposed twins without PTSD (ExP-). The left graph presents beta weight for left insula (-32, 2, 12) activation for early conditioning. The right graph presents beta weight for left insula (-32, 2, 12) activation separately for early CS+ and early CS-.

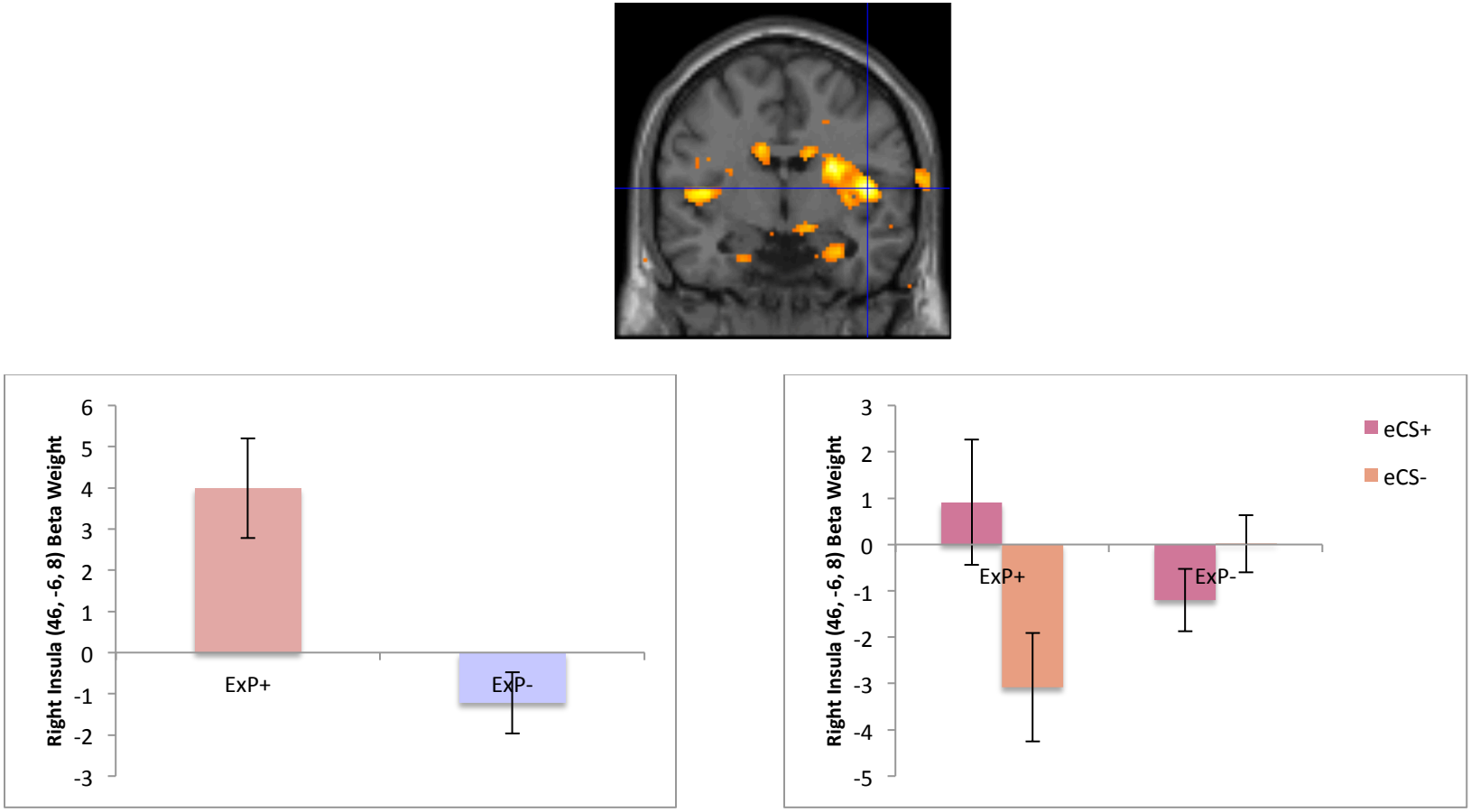

Figure 7. Right insula (MNI: x=46, y=-6, z=8) activation for early conditioning in combat-exposed twins with PTSD (ExP+) and combat-exposed twins without PTSD (ExP-). The left graph presents beta weight for right insula (46, -6, 8) activation for early conditioning. The right graph presents beta weights for right insula (46, -6, 8) activation separately for early CS+ and early CS-.

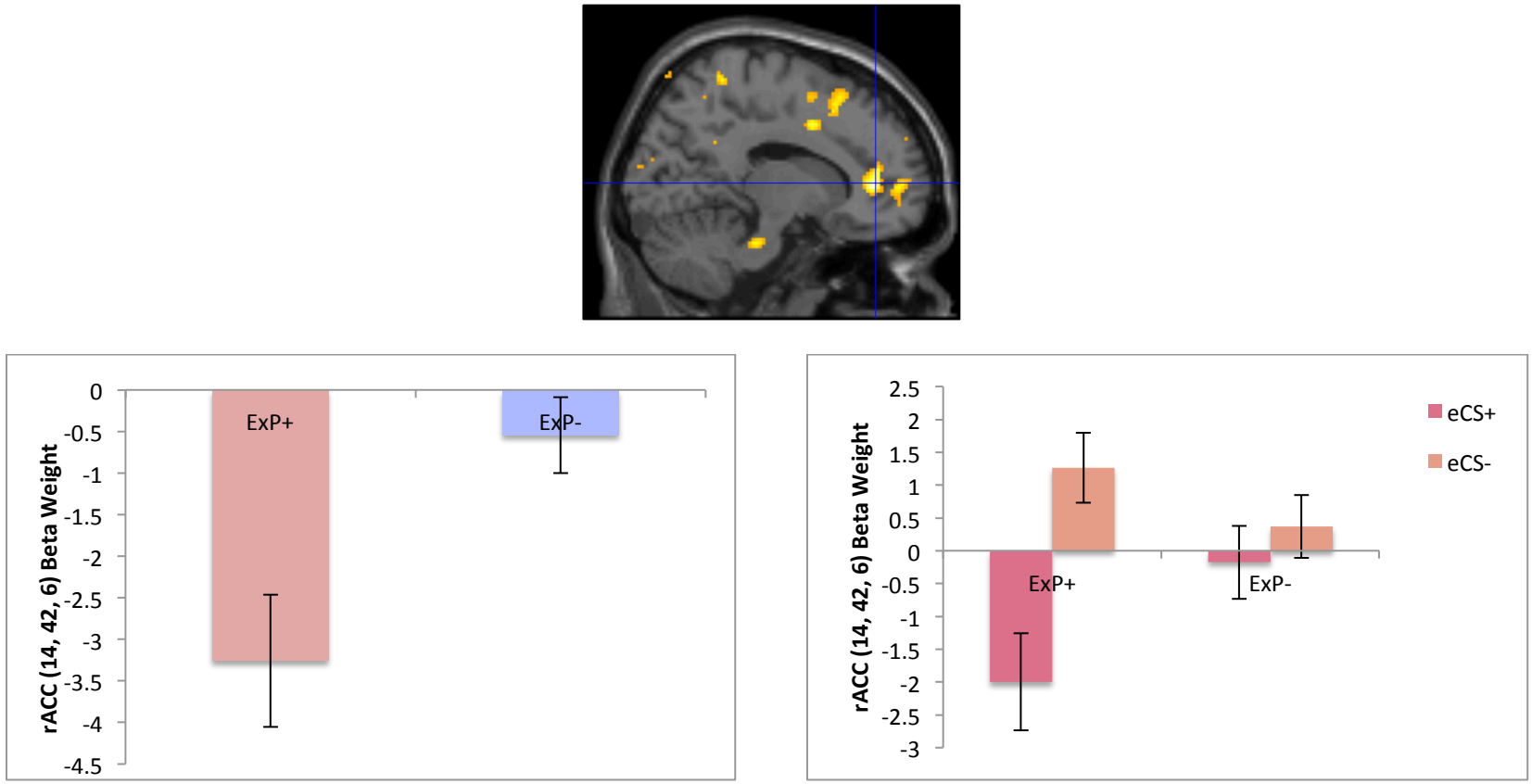

Figure 8. Rostral anterior cingulate cortex (rACC) (MNI: x=14, y=42, z=6) activation for early conditioning in combat-exposed twins with PTSD (ExP+) and combat-exposed twins without PTSD (ExP-). The left graph presents beta weight for rACC (14, 42, 6) activation for early conditioning. The right graph presents beta weights for rACC (14, 42, 6) activation separately for early CS+ and early CS-.

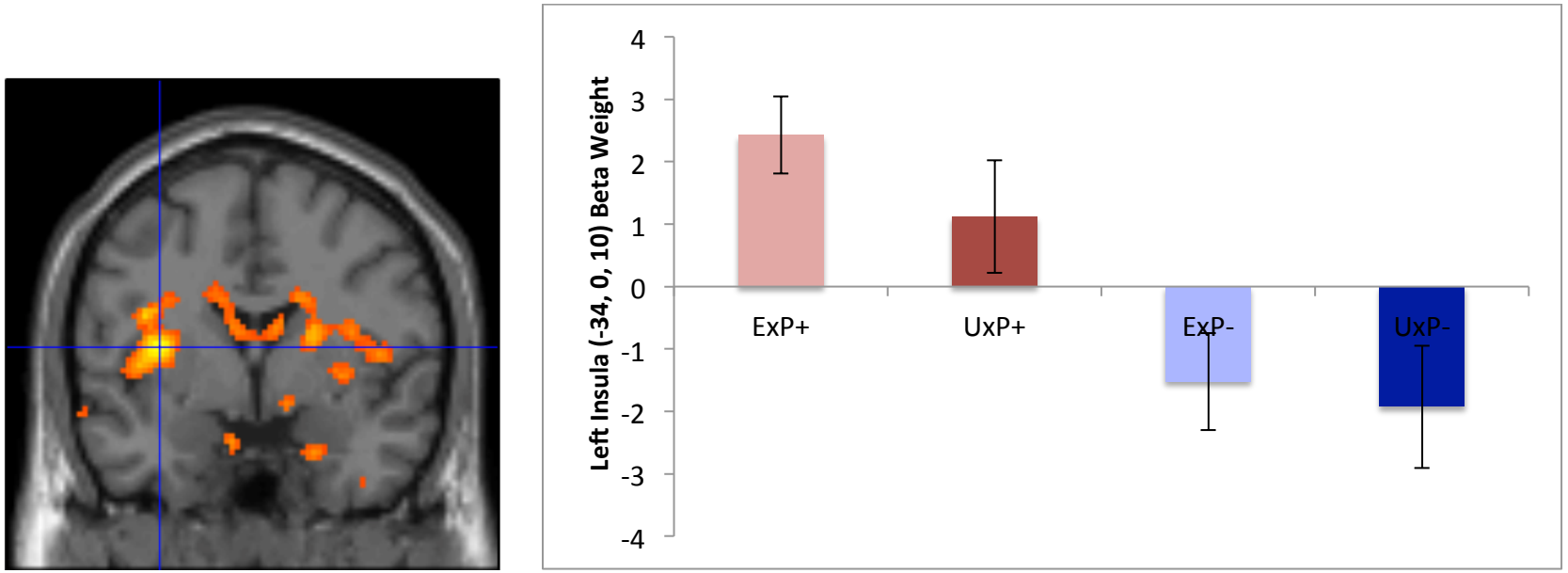

Figure 9. Left insula (MNI: x=-34 y=0, z=10) activation for early conditioning in PTSD twins and their co-twins and non-PTSD twins and their co-twins (main effect of diagnosis).

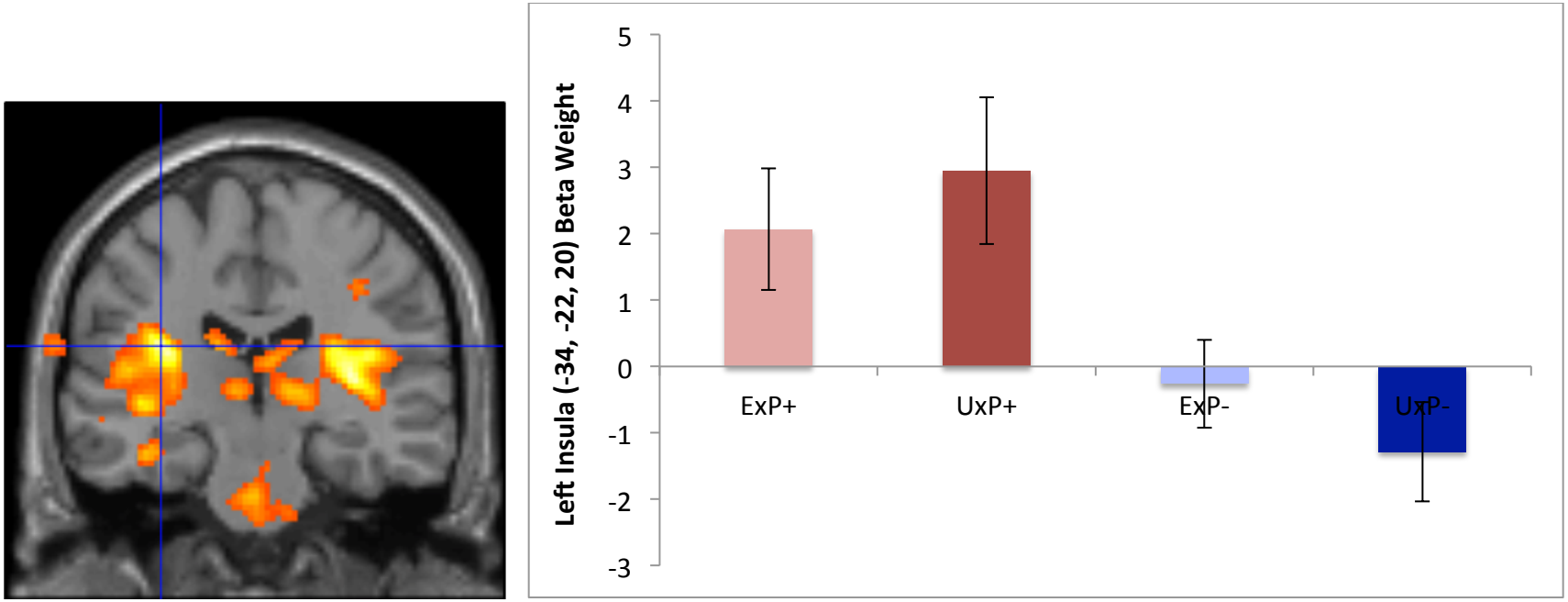

Figure 10. Left insula (MNI: x=-34, y=-22, z=20) activation for early conditioning in PTSD twins and their co-twins and non-PTSD twins and their co-twins (main effect of diagnosis).

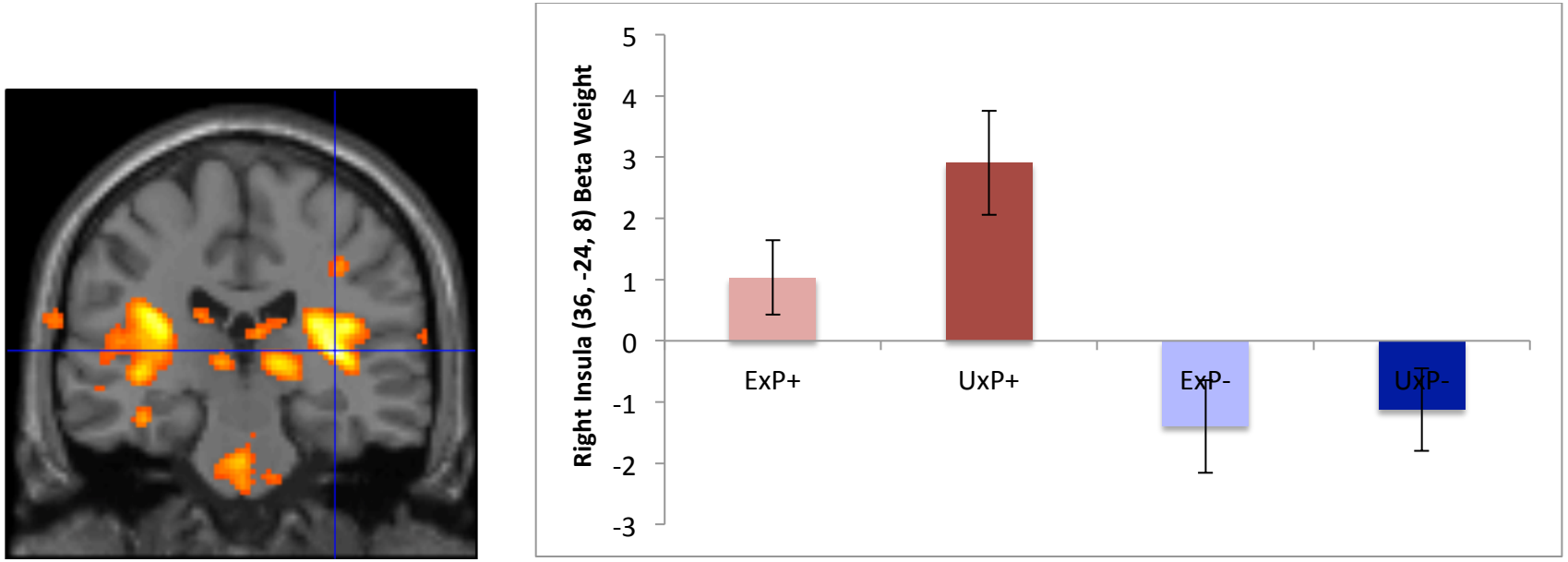

Figure 11. Right insula (MNI: x=36, y=-24, z=8) activation for early conditioning in PTSD twins and their co-twins and non-PTSD twins and their co-twins (main effect of diagnosis).

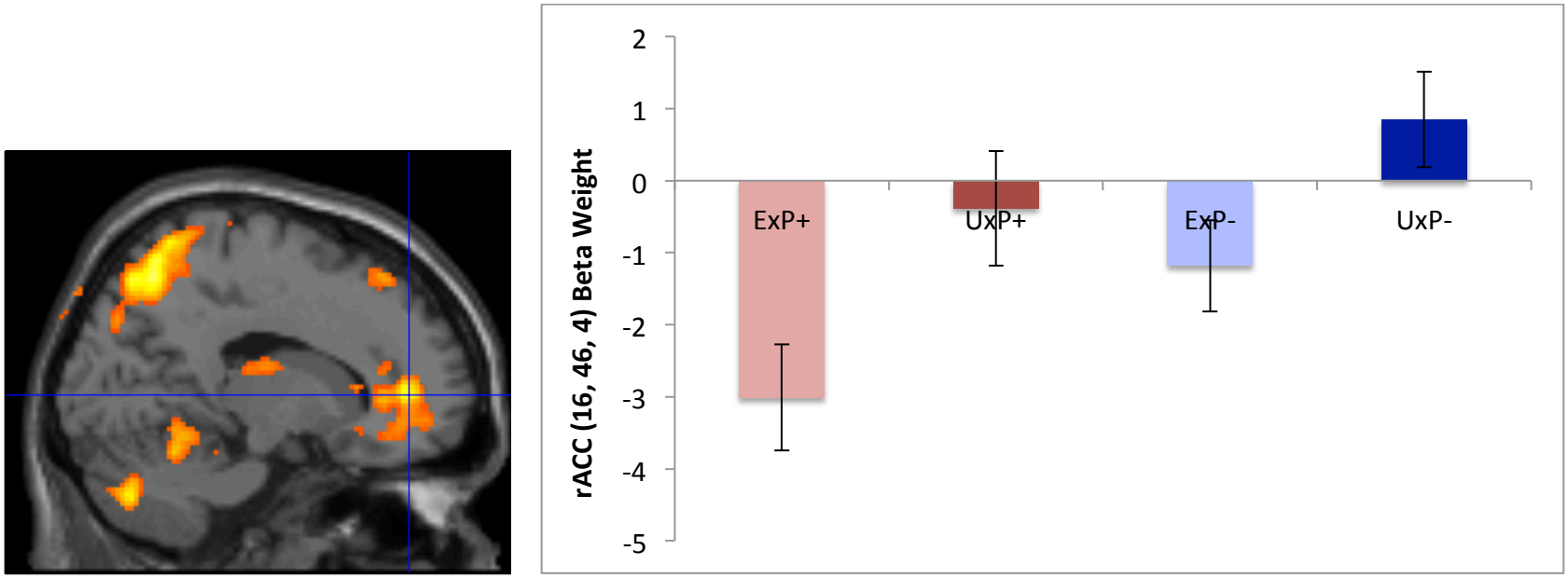

Figure 12. Rostral anterior cingulate cortex (rACC) (MNI: x=16, y=46, z=4) activation for early conditioning in the exposed twins and the unexposed twins (main effect of exposure).

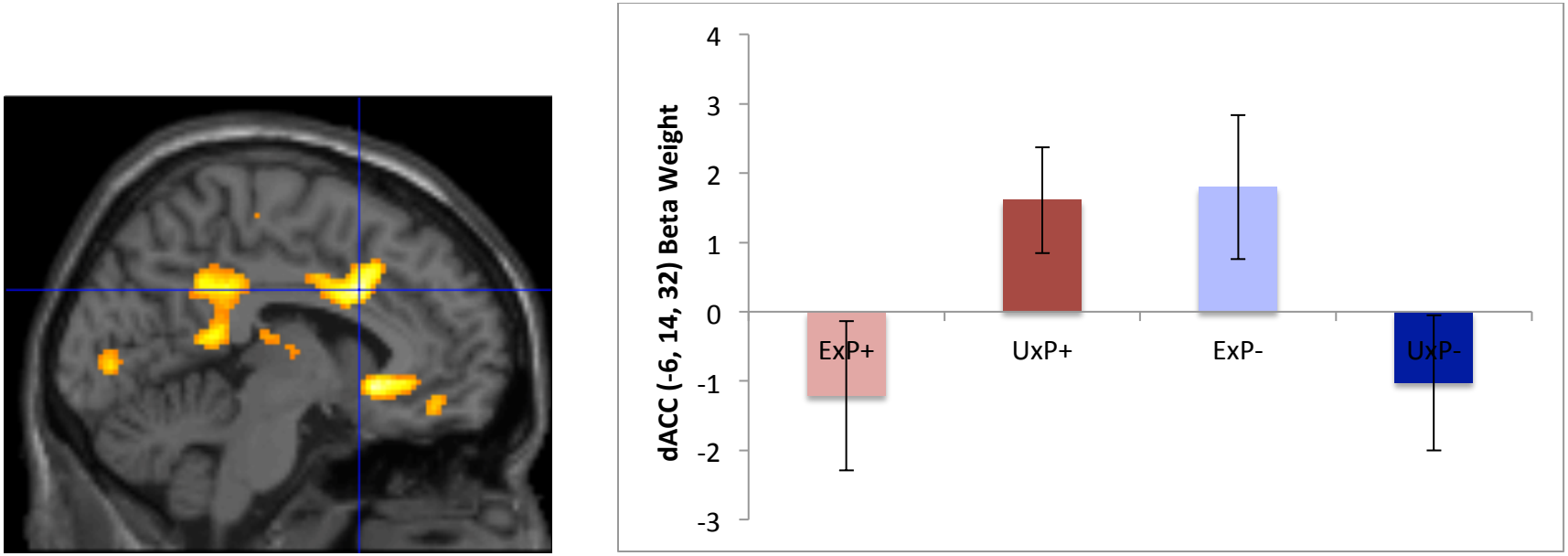

Figure 13. Dorsal anterior cingulate cortex (dACC) (MNI: x=-6, y=14, z=32) activation for early conditioning in the combat-exposed PTSD group (Diagnosis * Exposure interaction).

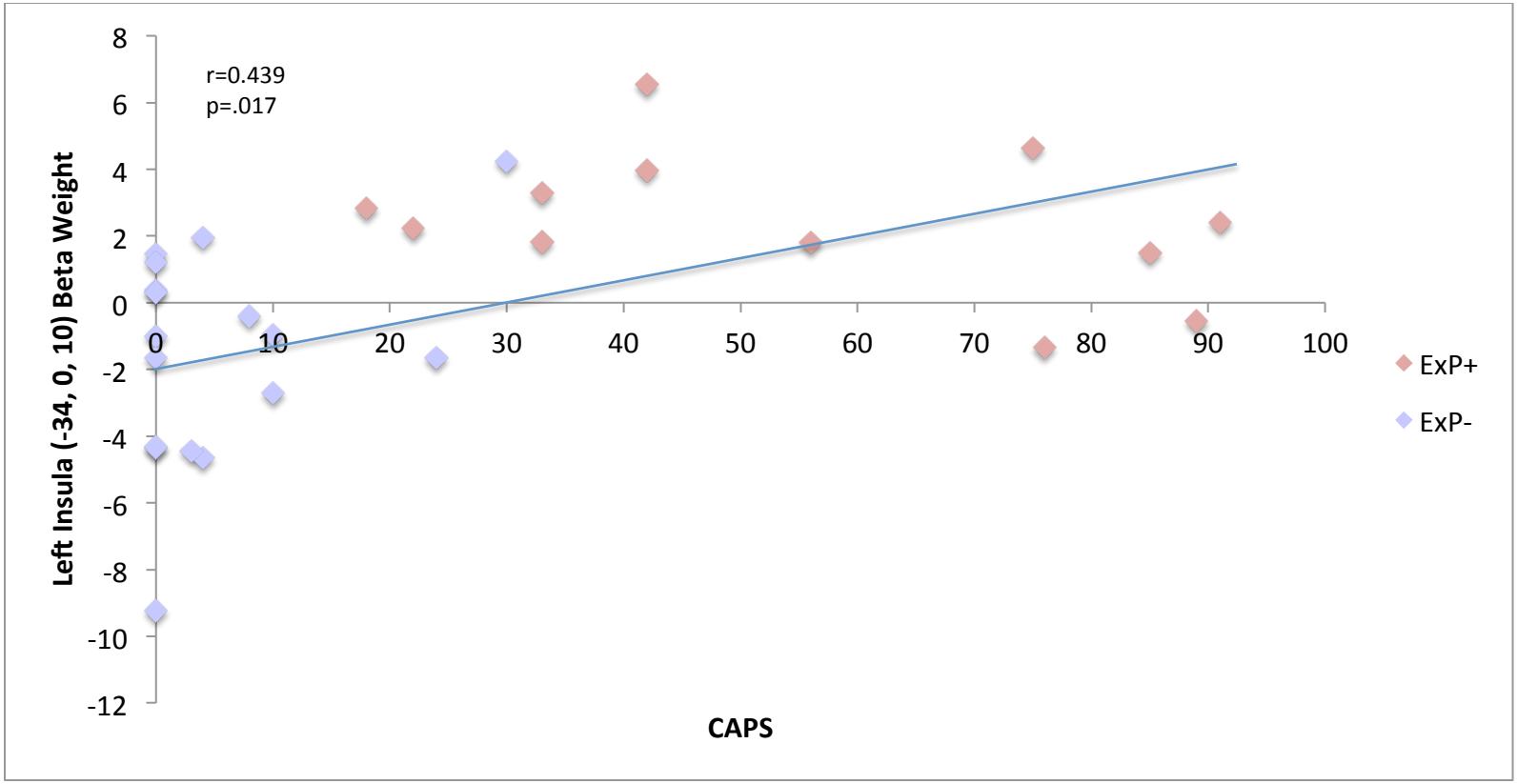

Figure 14. Correlation of combat-exposed twins' left insula (-34, 0, 10) activation for early conditioning with their own CAPS.

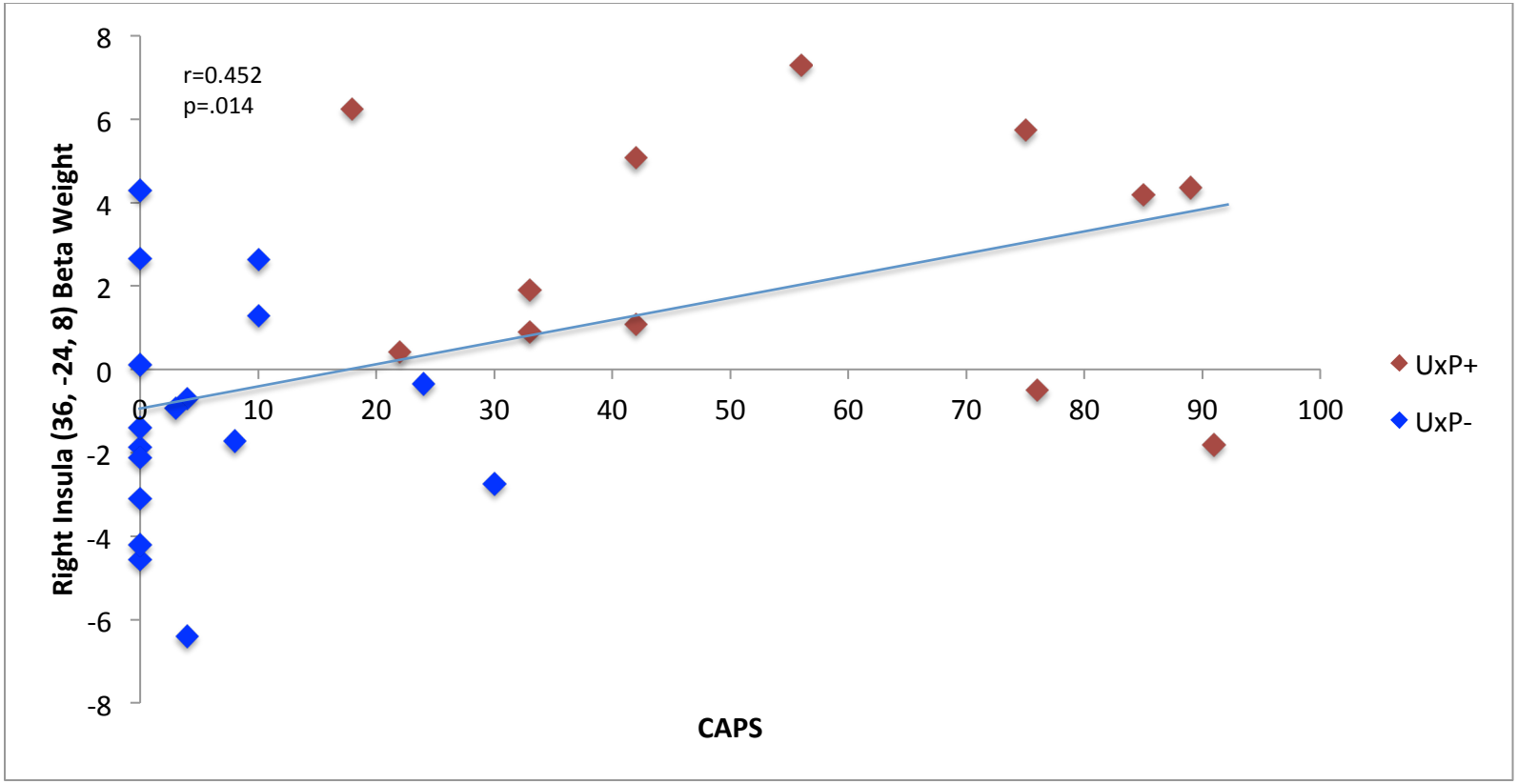

Figure 15. Correlation of combat-unexposed twins' right insula (36, -24, 8) activation for early conditioning with their exposed co-twins' CAPS.

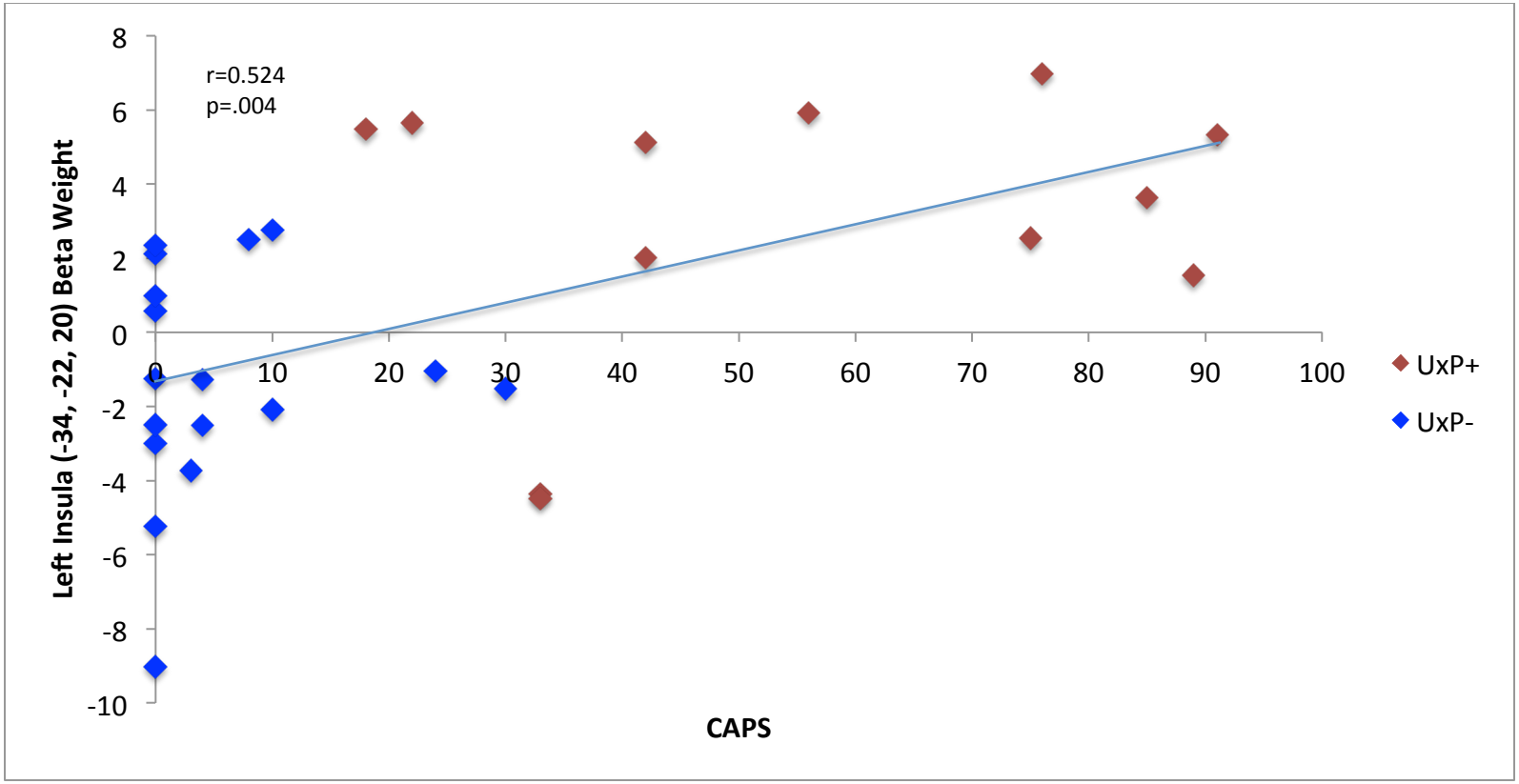

Figure 16. Correlation of combat-unexposed twins' left insula (-34, -22, 20) activation for early conditioning with their exposed co-twins' CAPS.

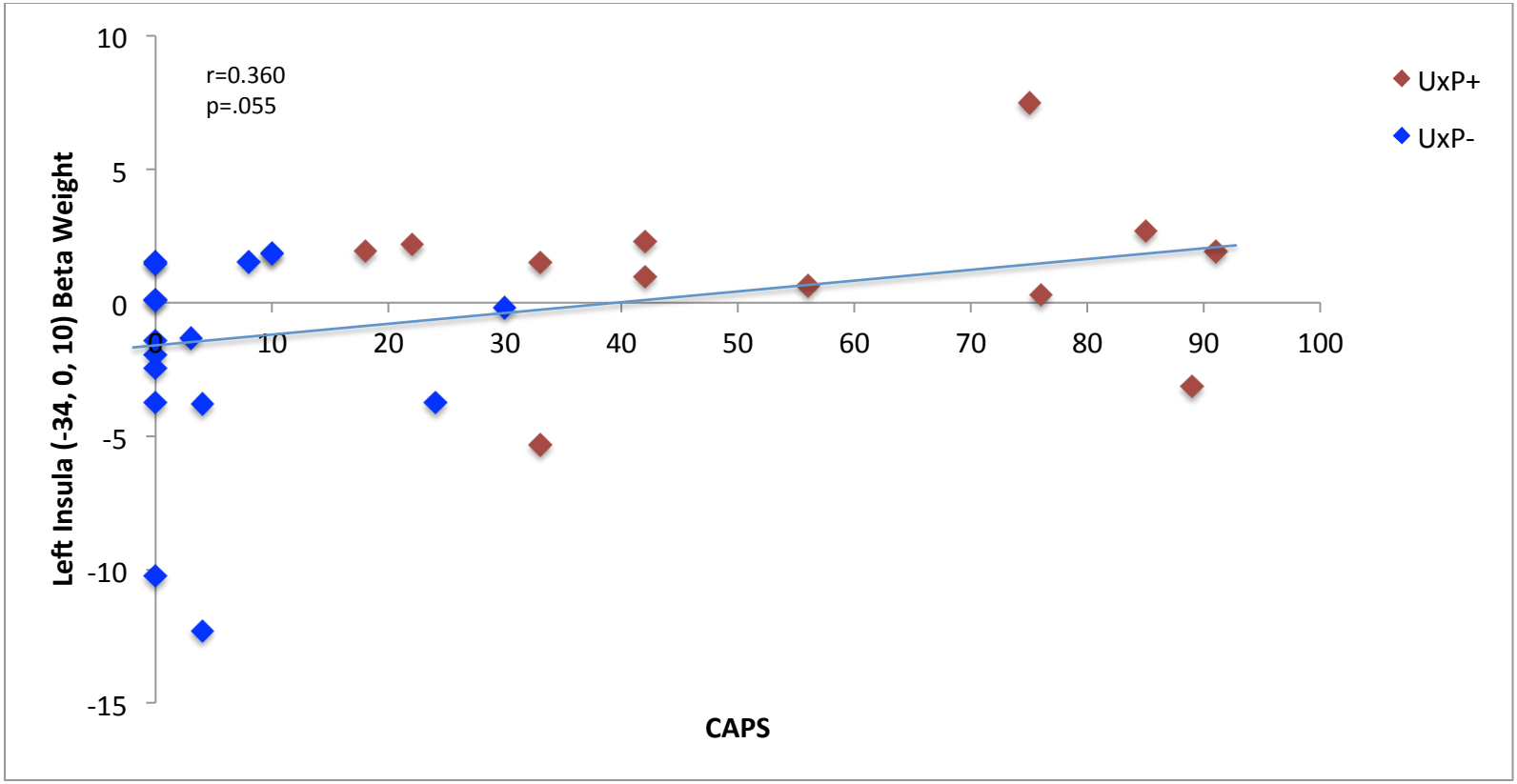

Figure 17. Correlation of combat-unexposed twins' right insula (-34, 0, 10) activation for early conditioning with their exposed co-twins' CAPS.

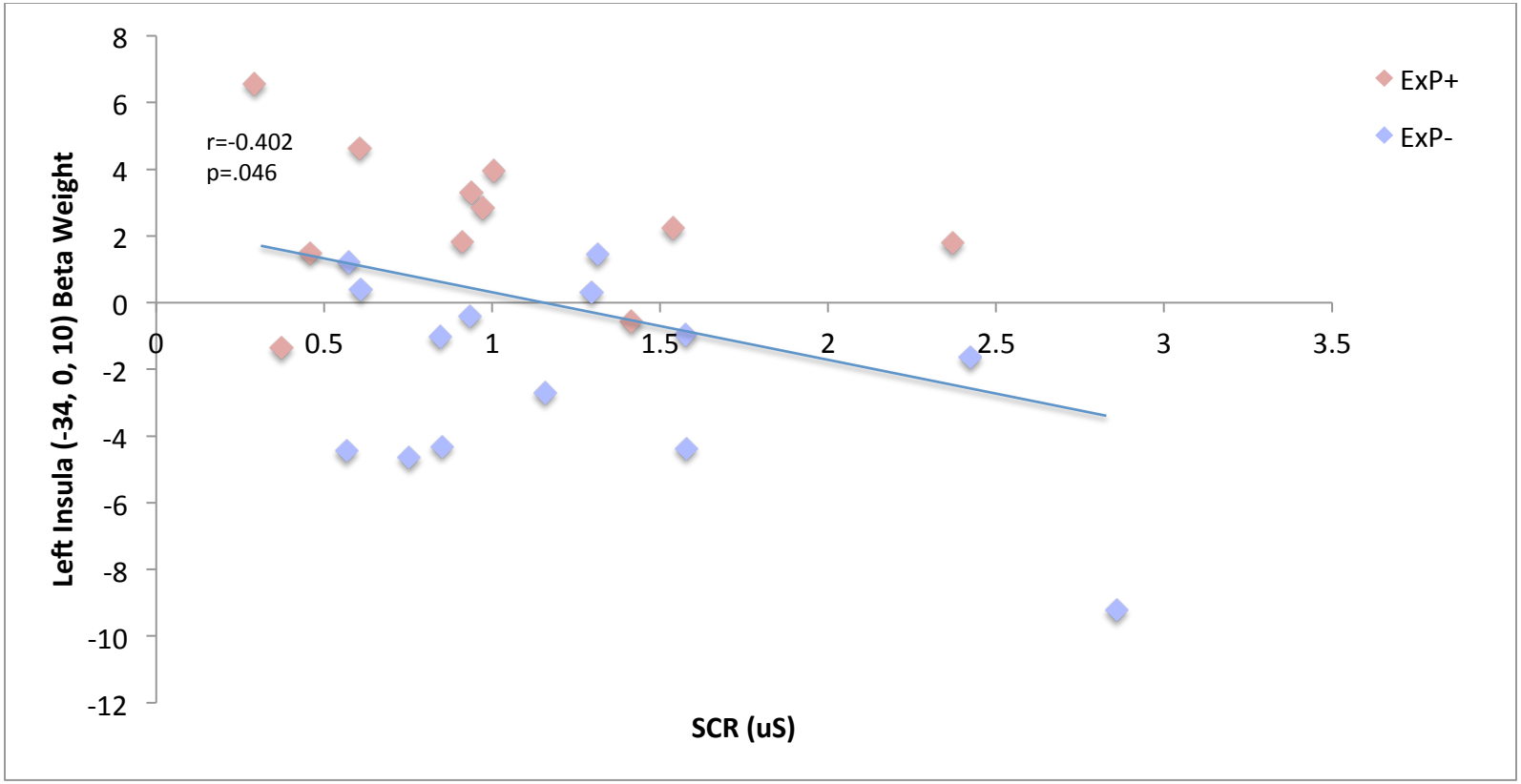

Figure 18. Correlation of combat-exposed twins' left insula (-34, 0, 10) activation for early conditioning with their own SCR for UCR to Shock. BOLD signal changes in the left insula (-34, 0, 10) during early conditioning in the combat-exposed twins were negatively correlated with their own SCR for the UCR to shock.

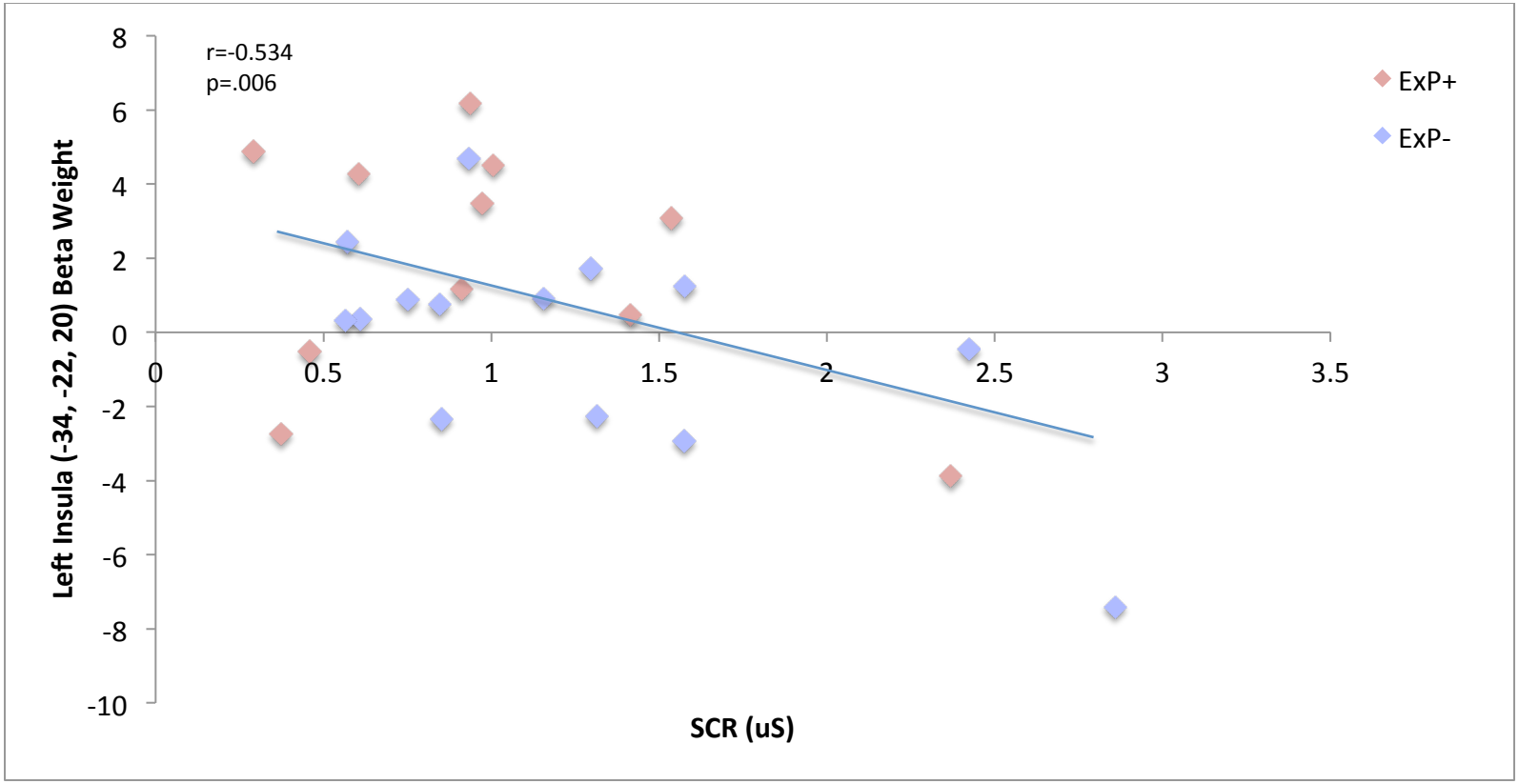

Figure 19. Correlation of combat-exposed twins' left insula (-34, -22, 20) activation for early conditioning with their own SCR for UCR to Shock. BOLD signal changes in the left insula (-34, -22, 20) during early conditioning in the combat-exposed twins were negatively correlated with their own SCR for the UCR to shock.

Appendix A

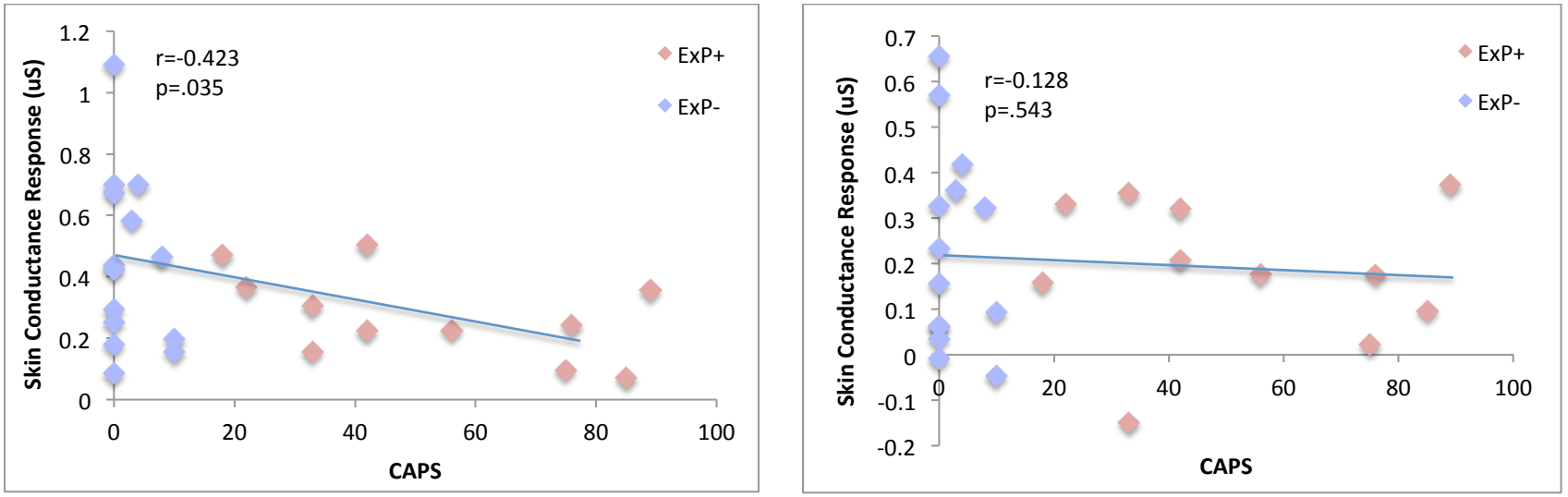

Figure A1. Correlation of combat-exposed twins' SCR for early CS+ and early CS- with their own CAPS. The left graph presents the correlation of combat-exposed twins' SCR during early CS+ trials with their own CAPS. The right graph presents the correlation of combat-exposed twins' SCR during early CS- trials with their own CAPS.

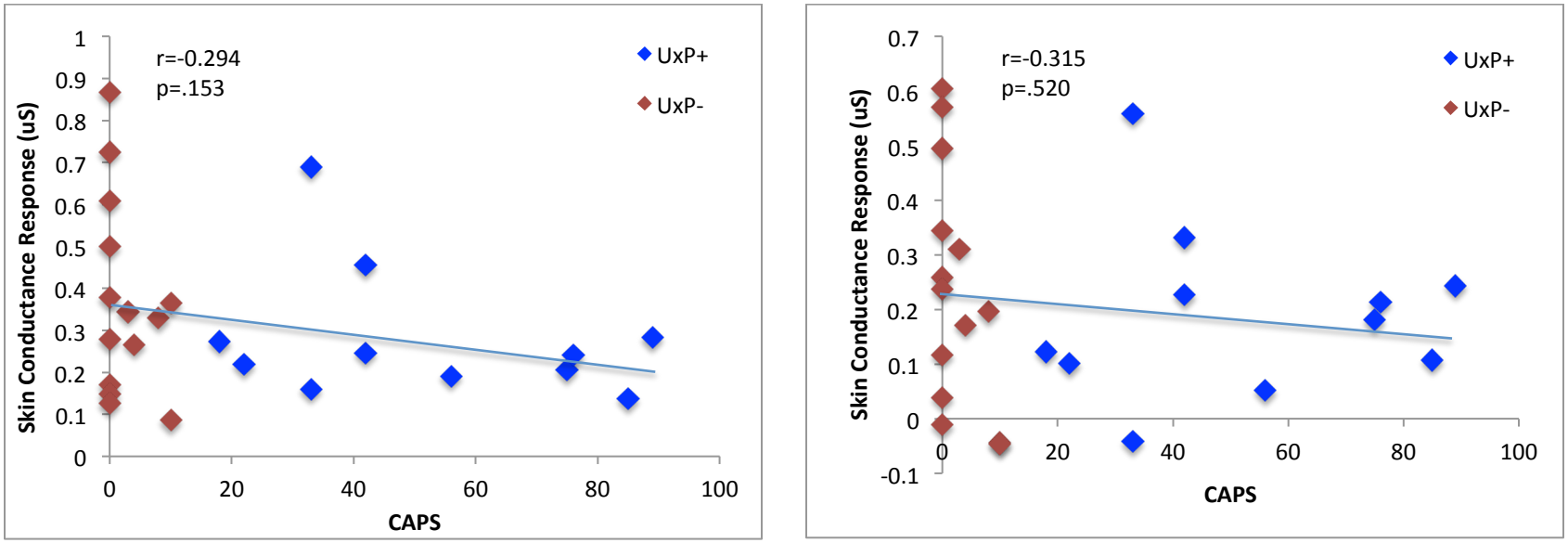

Figure A2. Correlation of combat-unexposed twins' SCR for early CS+ and early CS- with their combat-exposed co-twins' CAPS. The left graph presents the correlation of combat-unexposed twins' SCR during early CS+ trials with their combat-exposed co-twins' CAPS. The right graph presents the correlation of combat-unexposed twins' SCR during early CS- trials with their combat-exposed co-twins' CAPS.

Appendix B

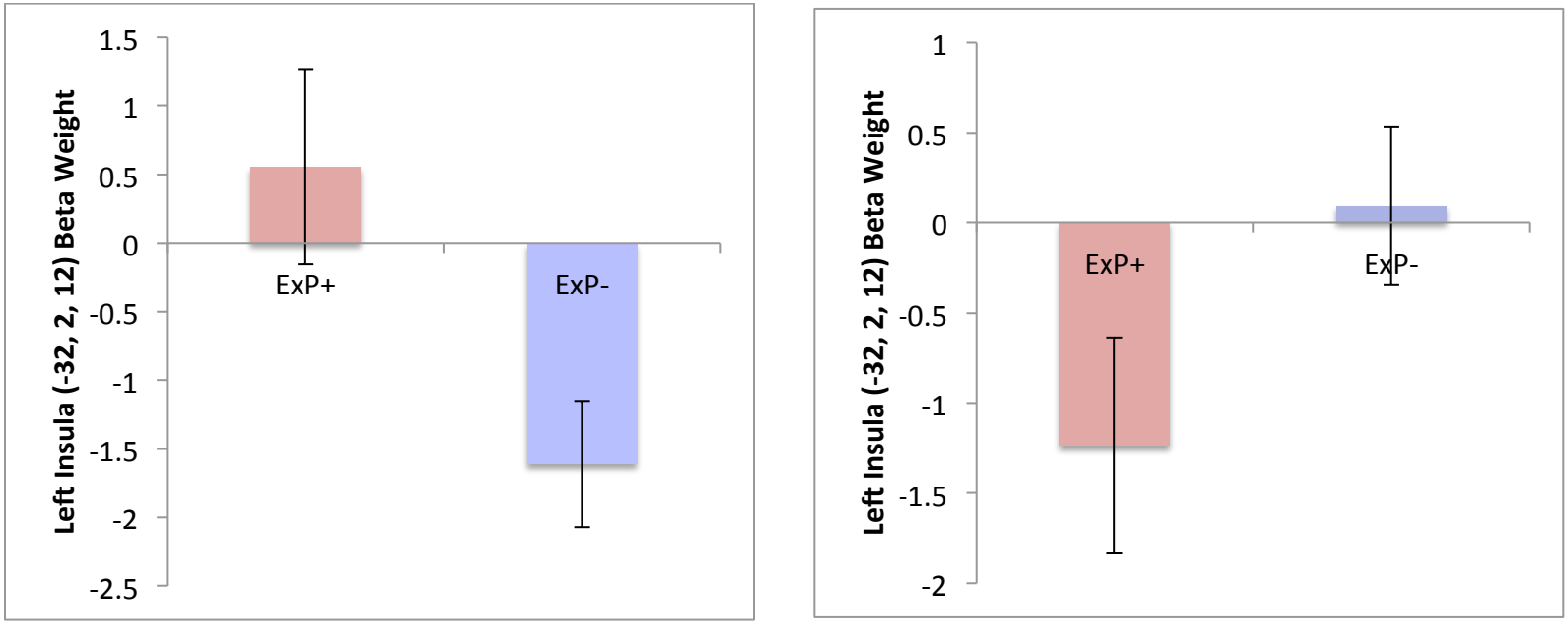

Figure B1. Left insula (MNI: x=-32, y=2, z=12) activation for early CS+ trials (left graph) and early CS- trials (right graph) in combat-exposed twins with PTSD (ExP+) and combat-exposed twins without PTSD (ExP-).

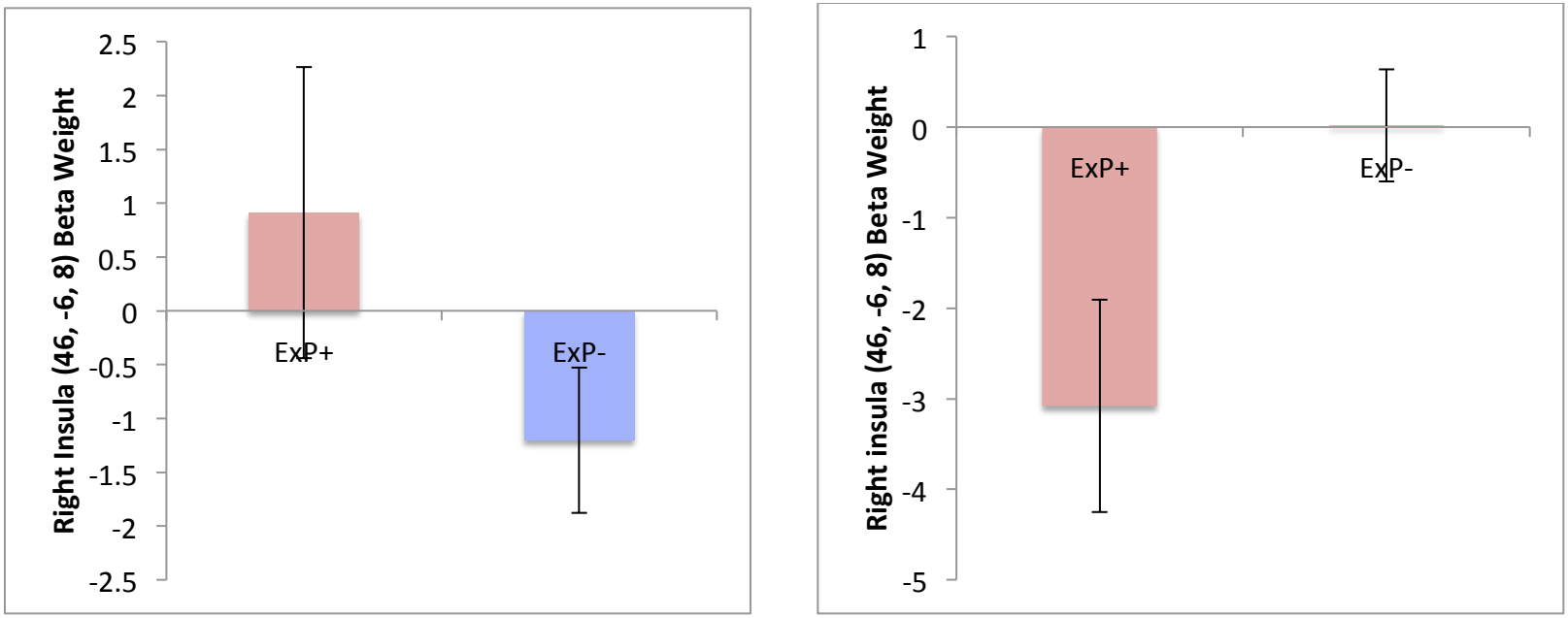

Figure B2. Right insula (MNI: x=46, y=-6, z=8) activation for early CS+ trials (left graph) and early CS- trials (right graph) in combat-exposed twins with PTSD (ExP+) and combat-exposed twins without PTSD (ExP-).

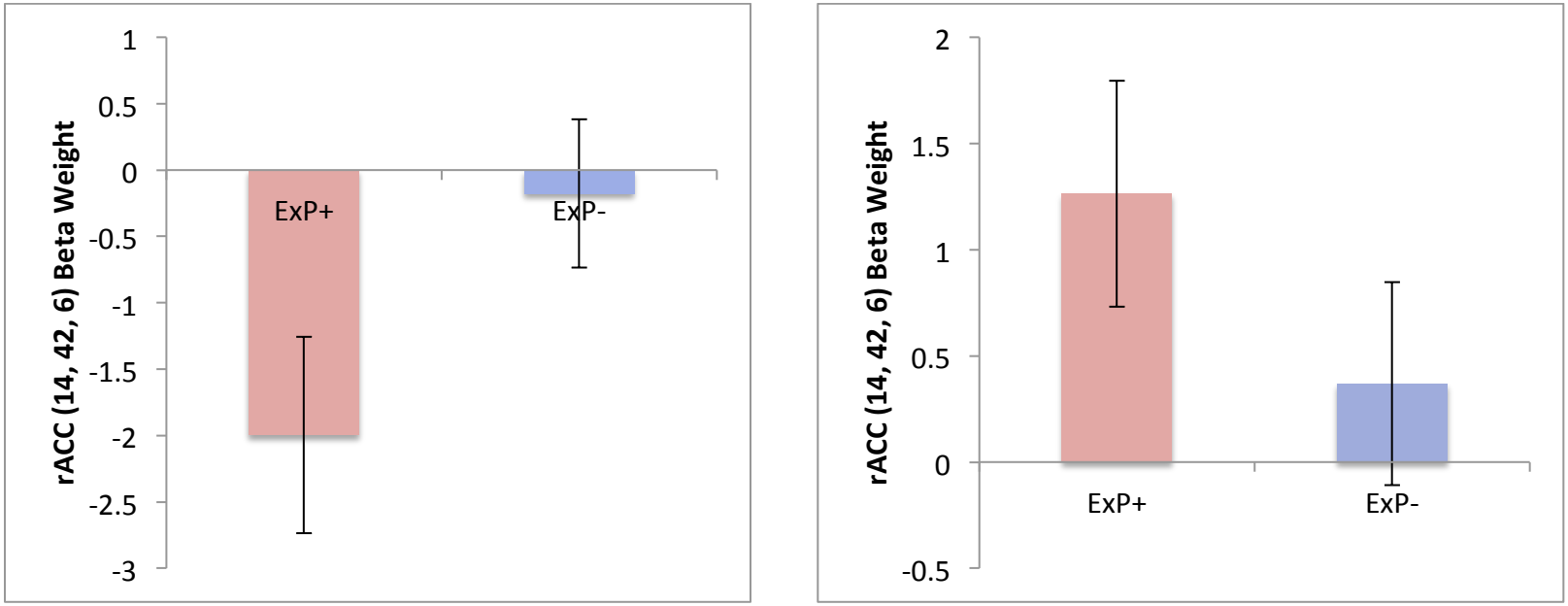

Figure B3. Rostral anterior cingulate cortex (rACC) (MNI: x=14, y=42, z=6) activation for early CS+ trials (left graph) and early CS- trials (right graph) in combat-exposed twins with PTSD (ExP+) and combat-exposed twins without PTSD (ExP-).

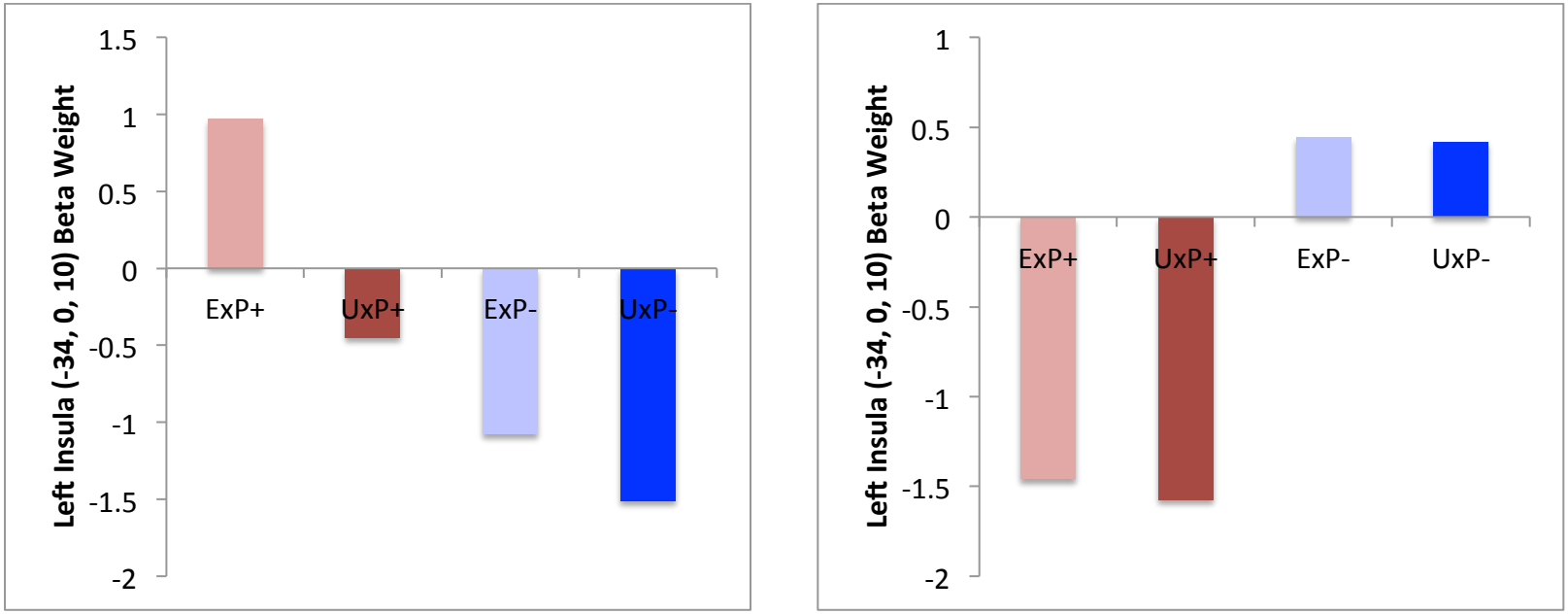

Figure B4. Left insula (MNI: x=-34 y=0, z=10) activation for early CS+ trials (left graph) and early CS- trials (right graph) in PTSD twins and their co-twins and non-PTSD twins and their co-twins (main effect of diagnosis).

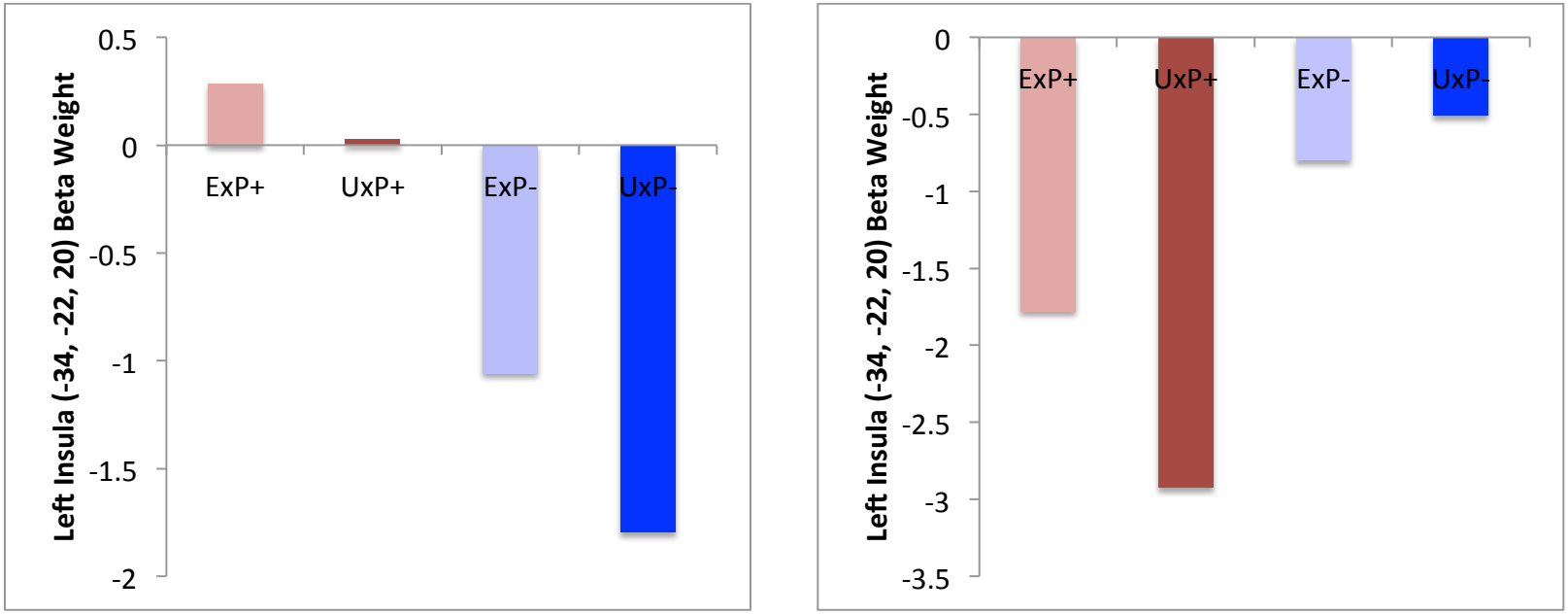

Figure B5. Left insula (MNI: x=-34, y=-22, z=20) activation for early CS+ trials (left graph) and early CS- trials (right graph) in PTSD twins and their co-twins and non-PTSD twins and their co-twins (main effect of diagnosis).

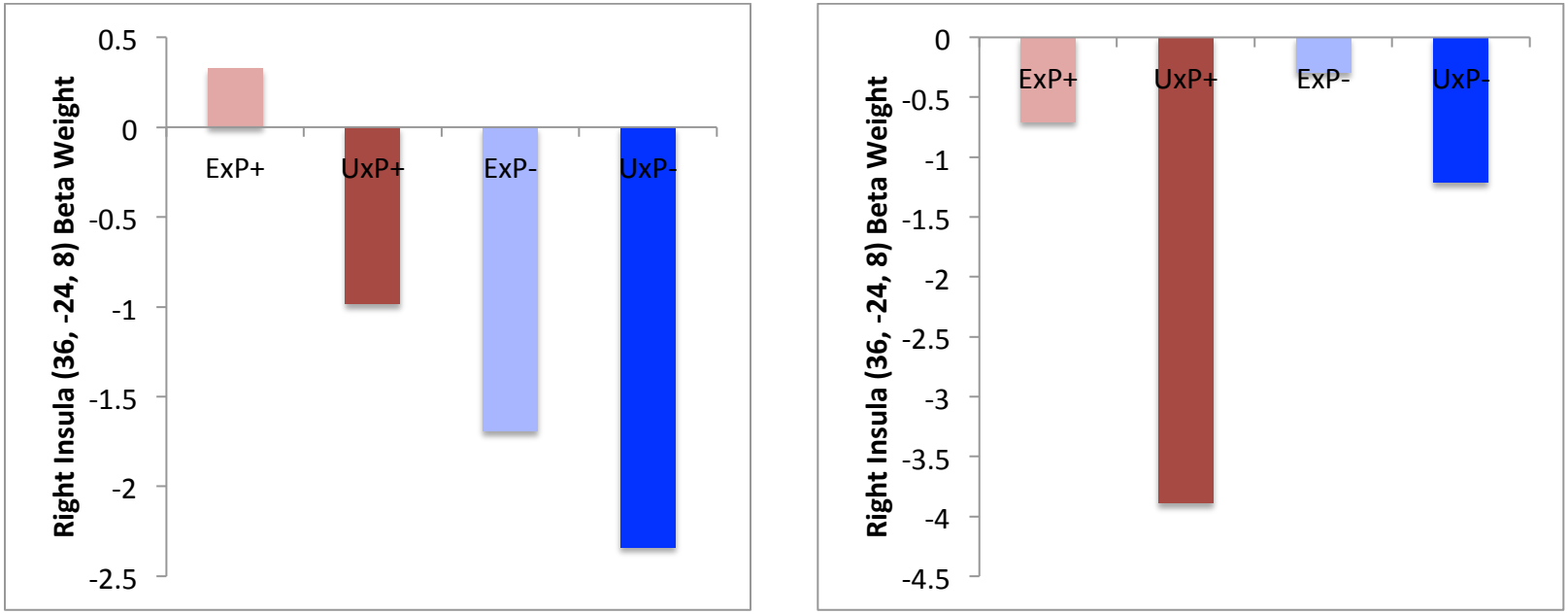

Figure B6. Right insula (MNI: x=36, y=-24, z=8) activation for early CS+ trials (left graph) and early CS- trials (right graph) in PTSD twins and their co-twins and non-PTSD twins and their co-twins (main effect of diagnosis).