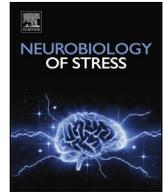




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Differences in frontal and limbic brain activation in a small sample of monozygotic twin pairs discordant for severe stressful life events



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ABSTRACT

Monozygotic twin pairs provide a valuable opportunity to control for genetic and shared environmental influences while studying the effects of nonshared environmental influences. The question we address with this design is whether monozygotic twins selected for discordance in exposure to severe stressful life events during development (before age 18) demonstrate differences in brain activation during performance of an emotional word-face Stroop task. In this study, functional magnetic resonance imaging was used to assess brain activation in eighteen young adult twins who were discordant in exposure to severe stress such that one twin had two or more severe events compared to their control co-twin who had no severe events. Twins who experienced higher levels of stress during development, compared to their control co-twins with lower stress, exhibited significant clusters of greater activation in the ventrolateral and medial prefrontal cortex, basal ganglia, and limbic regions. The control co-twins showed only the more typical recruitment of frontoparietal regions thought to be important for executive control of attention and maintenance of task goals. Behavioral performance was not significantly different between twins within pairs, suggesting the twins with stress recruited additional neural resources associated with affective processing and updating working memory when performing at the same level. This study provides a powerful glimpse at the potential effects of stress during development while accounting for shared genetic and environmental influences.

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1. Introduction

A wide range of evidence shows that exposure to acute and chronic stress is associated with impairments in flexible, goal-directed cognition (Arnsten, 2009; Casey et al., 2010; Lupien et al., 2009). Here, we compare patterns of brain activation in a small and rare sample of young adult monozygotic twins (i.e., identical twins) who were discordant in exposure to severe stressful life events during development (before age 18). Specifically, the twin pairs in this study were selected such that one twin reported at least two severe stressful events during adolescence while their control co-twin had no events or only a moderate event, but were reared together and therefore shared some environmental influences such as schooling and socioeconomic factors.

Functional magnetic resonance imaging (fMRI) was used to assess blood-oxygen-level dependent (BOLD) alterations during an emotional word-face Stroop task, which is a variant of the classic color-word Stroop task. Therefore, this study selectively highlights alterations in brain activation, that are specific to nonshared environmental influences including their discordant exposure to severe life stress, during a task that requires flexible, goal-directed cognition to overcome emotional conflict.

1.1. Executive function and stress

Executive Function (EF) is a broad term used to describe flexible, goal-directed cognition. It is measured by a range of tasks assessing the ability to maintain a goal, shift between goals, update working memory, and more complex abilities such as planning (Miyake et al., 2000; Shallice et al., 1996; Stuss and Alexander, 2007). Behavioral, neuroimaging, and pharmacological manipulations in humans and animals, as well as controlled animal experiments,

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have all shown stress-related behavioral impairments on a range of tasks measuring flexible, goal-directed cognition (Arnsten, 2009; Dedovic et al., 2009; Holmes and Wellman, 2009; Ulrich-Lai and Herman, 2009). The Stroop task, a common measure of EF, requires goal-maintenance when there is conflicting or incongruent goal-irrelevant information (MacLeod, 1991). The objective of the emotional word-face Stroop task used in this study is to respond to a word positioned on a face with an emotional expression, which on some trials is congruent (ex. “Happy” on a happy face), and on other trials is incongruent with that expression (ex. “Happy” on a sad face). Hence, the emotional word-face Stroop task retains the features of a traditional Stroop task, while adding a social-emotional distraction (emotional faces) that might be particularly challenging to ignore for people who have experienced stress during development (Carretié, 2014; Cisler and Koster, 2011; Hayes et al., 2012).

Neuroimaging evidence is consistent with behavioral stress-related deficits in EF performance. More specifically, it shows that exposure to stress impairs or alters activity of the prefrontal cortex (PFC) during EF tasks (See reviews: Arnsten, 2009; Dedovic et al., 2009). For example, in one well-controlled neuroimaging study, behavioral deficits and altered connectivity within the frontoparietal network were found in young adults during an EF shifting task after one month of psychosocial stress (preparing for a medical licensing exam), compared to a matched control group (Liston et al., 2009). This study implicated EF as a specific stress-related impairment, as it did not find impaired performance on a non-EF task matched for cognitive difficulty. Furthermore, in the absence of psychosocial stress one-month later, frontal-parietal activity and performance returned to pre-stress levels. This study demonstrates that EF changes due to acute stress seem to be reversible and that deficits may be specific to EF (Liston et al., 2009). One question that remains is whether severe stress during sensitive developmental periods and/or chronic stress may have longer-term implications. The goal of this study was to select monozygotic twin pairs solely on discordance in stress during development and use these differences in severe stressful life events as a predictor of brain activation patterns during an EF task in young adulthood.

1.2. Discordant twin studies

Discordant twin studies are particularly powerful for determining nonshared environmental effects in the face of familial similarity. While there are some hints of stress related functional and structural changes in the brain, the nature of this relationship in humans is less clear. In a well known case-control study, MZ twins were selected such that they were discordant in Vietnam combat exposure. The combat exposed twins were selected with and without Post Traumatic Stress Disorder (PTSD). Smaller hippocampal volume was shared between twin pairs who were discordant for PTSD and combat exposure as compared to the twin pairs neither of whom had PTSD and were discordant for combat exposure (Gilbertson et al., 2002). This study further showed that in the combat exposed twin with PTSD, smaller hippocampal volume was associated with the increased likelihood of developing a remittent form of PTSD and impaired neurocognitive performance on a hippocampal-dependent, configurable cue-processing task, thereby providing evidence that this variation may confer a vulnerability to stress (Gilbertson et al., 2002, 2006, 2007). These findings emphasize that the integrity of this structure may influence sensitivity to stress and that there is a familial component, which may either reflect a genetic vulnerability or shared experiences prior to Vietnam.

In another study, MZ twins with higher depression and anxiety showed smaller hippocampal volume when compared to their

control co-twins, suggesting that nonshared environmental influences accounted for this difference (de Geus et al., 2007). Specifically, monozygotic twins at least 0.5 standard deviations above the average of a 9-year longitudinal assessment of depression and anxiety compared to their discordant control co-twins (at least 2 SD below their co-twin) showed reduction in hippocampal volume. Although only exploratory, de Geus et al. (2007) also found that the MZ twins with higher depression and anxiety scores reported a greater number of stressful events. In the Vietnam-era PTSD study, PTSD twins also reported a greater number of stressful events compared to both their control co-twins without combat exposure and the unrelated combat exposed non-PTSD twins (Gilbertson et al., 2006). Additional research is needed to understand the potential three-way link between stress, the hippocampus, and symptoms associated with PTSD, depression and anxiety.

The anterior cingulate cortex (ACC) is another region associated with familial effects and PTSD. Specifically, combat-exposed veterans with PTSD and their non-combat exposed co-twins showed greater activation in the dorsal ACC compared to twin pairs without PTSD (Shin et al., 2011), which is similar to familial effects found with the volume of the hippocampus. This differential activation was observed while participants were completing a multisource interference task, which consisted of a condition in which the identity of the target number did not match the position on the button box and was therefore conflicting. The present study may help to clarify whether exposure to stress during development might account for some of the variation in limbic and prefrontal alterations seen in PTSD, depression, and anxiety.

2. Present study

The present study utilized functional brain data in MZ twins discordant for stress during development. The emotional word-face Stroop task was used to assess the degree to which activation in the frontoparietal network (including lateral and medial PFC), basal ganglia and limbic regions such as the amygdala and hippocampus are differentially associated with life events in the stress-discordant MZ twins. This task was designed to measure overall goal maintenance in the face of emotionally distracting information, as well as more specific contrasts that compare incongruent trials on which the word and facial expression are in conflict, to congruent trials on which they are the same (Etkin et al., 2011; Hadwin et al., 2009). Twin pairs were selected such that one twin had at least 2 severe events while their control co-twin had at most 1 minor event during adolescence. Selection was focused on events that occurred during adolescence or began in childhood but continued into adolescence. However, due to the correlation between stress during childhood and adolescence we were unable to select twins with only stress during adolescence and thus refer to the stress as developmental. By selecting reared-together, MZ twins who differ in exposure to stress and by using a task that incorporates emotional faces as distractors, this study aims to assess stress-related differences in the neural responses involved in executive control over distracting emotional stimuli, controlling for shared genetics and environment.

We hypothesize that twins exposed to severe stress during development will show greater activation in medial PFC and limbic regions associated with processing emotional or evaluative information, while their co-twins with low stress will show more typical frontoparietal activation associated with implementing cognitive control (Fedorenko et al., 2013). Specifically, previous work has shown that the lateral PFC, and dorsal regions of the ACC are recruited during EF tasks such as the color-word Stroop task and versions of the emotional Stroop task (Andrews-Hanna et al., 2011; Banich, 2009; Etkin et al., 2011; Mohanty et al., 2007; Preston and

Stansfield, 2008). Medial regions of the PFC, the basal ganglia, and limbic regions such as the hippocampus and amygdala are recruited in the processing of emotional information and activated during stress (Dedovic et al., 2009; Ulrich-Lai and Herman, 2009; McEwen et al., 2015). We hypothesize that the twins exposed to high levels of severe stress will exhibit decreased activation of the dorsolateral PFC and increased activation of the medial PFC, basal ganglia, amygdala and hippocampus throughout the task. Specifically, we tested this hypothesis through whole brain analyses and extracted percent signal change from a priori regions drawn from a review of the effects of stress on the brain including the hippocampus, ACC of the medial prefrontal cortex, amygdala, and thalamus (see Table 1 of Ulrich-Lai and Herman, 2009). We also hypothesize that the control co-twins will show greater activation of frontoparietal regions during the (I-C) contrast compared to their co-twins with stress. The incongruent minus congruent contrast (I-C) is thought to capture the flexibility of EF performance based on the demands of the task. This contrast has shown greater activation specific to incongruent blocks in frontoparietal regions and ACC (Banich et al., 2000). By selecting MZ twins discordant in exposure to stress, this study aims to characterize brain activation in twins exposed to severe stress during development compared to their control co-twins without exposure to severe stress.

3. Methods

3.1. Participants and selection criteria

Data from a community twin sample was utilized for the selection of 20 monozygotic twins (10 twin pairs). This sample has been followed longitudinally since early adolescence (Rhea et al., 2013). Monozygotic (MZ) twins were selected from a sample of 290 MZ twin pairs, based solely on count of severe stressful life events that occurred before the age of 18, as retrospectively reported in young adulthood (mean age = 25.5). Participants were in their mid-twenties to early thirties at the time of neuroimaging (mean age = 30.2). One twin pair recruited into the study was excluded due to motion, leading to a final N of 18 twins (44% female). Of these twin pairs, behavioral data from two twin pairs were not included in the accuracy and reaction time analyses due to button box malfunctions that led to missing data for some conditions. Six additional pairs were recruited such that they varied in concordance and discordance in stress exposure but were not included in this study since they did not fit the discordant-control design. Although this sample is relatively small, the use of discordant twin designs is highly endorsed as a powerful design (van Dongen et al., 2012; Zwiijnenburg et al., 2010) with the potential to be more powerful at finding replicable effects than larger samples that vary on a range of genetic backgrounds and life experiences. However, due to the small sample size, this study may not have the power to detect moderate or variable effects.

The Life Events Checklist – a measure of accidents, major life events, illness, deaths, and unemployment (Gray et al., 2004), and the CARI-Questionnaire – a measure of neglect, emotional, physical, and sexual abuse, were used for counts of the number of severe stressful life events. Each event that occurred before age 18 from the ‘Whole Life’ section of the Life Events Checklist (19 severe events) and the CARI-Questionnaire (20 total questions) was counted as one event. These questionnaires ask about a variety of specific and memorable events such as having a person close to you die, getting fired, being intentionally burned or a time when you often went hungry, etc. Twin pairs were selected based on discordance in stress exposure such that the control co-twin had only 0–1 minor event beginning in adolescence (ages 9–17) and 0–2 minor events throughout development (ages 0–17), while their

twin with stress was selected such that they had experienced 2–7 severe events during development. The CARI-Q asks whether an event happened and then the first and last age it happened. Therefore, we were not able to separate childhood and adolescent events as some participants provided a wide range which overlapped between a childhood and adolescence or chose not to remember those details. However, an emphasis was placed on events beginning or continuing into adolescence and therefore all of the life events from the LEC occurred during adolescence and the majority of the CARI-Q events occurred during the pre and post pubertal stage in development (CARI-Q Mean age range: Twins with stress = 8.6–13.5; Control co-twins = 4.8–8.8). Twins with extreme counts of events were less likely to have a control co-twin with 0–1 minor events, therefore, there is a slight bias toward middle range scores on the CARI-Q. As selection was performed with only CARI-Q and LEC data, no exclusions were made. However, as part of the MRI prescreen, participant’s reported no Traumatic Brain Injuries, usage of potentially MRI altering medication, or MRI incompatibility.

An additional time point of stress questionnaires was collected after the neuroimaging portion of the study. The Life Stress and Controllability Index (LSCI) measured at the neuroimaging time point (See supplements) asks more general questions about types of stressors (to reduce participants having to recall details of events) during childhood (0–8 years), adolescence (9–17 years) and adulthood (18 + years). While the CARI-Q asks for a range of when the event occurred, the LSCI asks about 10 types of stressors during each developmental phase with an additional 5 questions in adulthood. The Perceived Stress Scale, a common measure of stress in the last month was also measured at the neuroimaging time point and is included in the supplements.

3.2. Emotional word-face stroop task

Neuroimaging was performed during a variant of the Stroop task, which is a task that is classically used to examine cognitive control. In the emotional word-face Stroop task used in this study, individuals were required to identify an emotional word while ignoring a face with an emotional expression that was either congruent or incongruent with the word (Fig. 1). Specifically, participants were instructed to respond according to a word superimposed on a face whose expression was on some trials congruent (e.g., the word “Happy” superimposed on a happy face), and on other trials incongruent (e.g., the word “Angry” superimposed on a happy face). The words “Happy”, “Sad”, “Angry”, “Neutral” and “Scrambled” were superimposed on faces with one of four emotional expressions (happy, sad, angry, neutral) or a neutral face image that was scrambled in Photoshop. These images were drawn from the Nimstim set of emotional face stimuli (Tottenham et al., 2009). Each stimulus was shown for a maximum of 1.5 s with a half second inter-trial fixation cross. At the time of the button press, whether correct or incorrect, the fixation cross was shown. To add a level of uncontrollability, trials with the word “Neutral” were always shown for 1.5 s regardless of button press.

A hybrid block design was used such that stimuli were first blocked by the emotion expression of the face, with blocks of 12 trials containing the same type of emotional expression (ex. all sad faces in the same block). Runs consisted of four blocks of each expression (angry, happy, sad and neutral) with the order counterbalanced. Within each of these blocks were 3 mini-blocks of 4 trials, counterbalanced by congruency. Scrambled faces were randomly interspersed in between emotional face trials at a rate of 1 trial per 4 trials. Fixation blocks, which lasted for 48 s, were placed in between runs for a total of 12 runs, which lasted a total of 24 min. This allowed for a robust block comparison between



Fig. 1. Face stimuli example used for the Emotional Word-Face Stroop Task. Incongruent and congruent emotional words were presented on Nimstim Face stimuli, blocked by emotion of face.

congruency of facial expression with word as well as different emotional facial expressions. The task order and stimulus response mapping remained consistent across participants in order to maintain the same experience between twin pairs.

3.3. Imaging protocol

Functional MRI data was collected using a Siemens 3T MRI scanner with a T2*-weighted gradient echo (repetition time = 2000 msec, echotime = 25 msec, flip angle = 73°, 38 slices parallel to the OFC line, thickness = 3 mm, 64 × 64 acquisition matrix, 3 mm × 3 mm resolution, in-plane field-of-view = 192 mm). Image preprocessing (spatial smoothing, intensity normalizing, high pass temporal filtering) and statistical analyses was performed within FSL (Jenkinson et al., 2012; FMRIB Software library, Oxford, UK, www.fmrib.ox.ac.uk). Images were run with the brain extraction tool (BET) to remove skull and other non-brain features. A high pass filtering cutoff of 100 s was used for temporal filtering and 5 mm full-width half maximum Gaussian Kernel was used for spatial smoothing. FMRIB's Improved Linear Model (FILM) was used for prewhitening before statistical analyses. Motion was corrected using the rigid body translation and rotation algorithm (MCFLIRT). Motion parameters for X, Y, and Z rotation and translation are included in the supplementary material (Table S1). There were no significant group differences in mean motion between the twins with stress and control co-twins for paired or group t-tests. One twin pair was excluded due to motion above 3 mm. Motion spikes above 2.5 mm were censored by creating motion regressors to exclude motion spikes for two participants.

3.4. Analyses

Group and paired sample t-tests were used to assess group differences (twins with stress compared to control co-twins) in reaction time and accuracy. The block design was used for analyses of the fMRI data using FMRI Expert Analysis Tool (FEAT). Group and paired t-tests for our contrasts of interest (Task - Fixation and Incongruent - Congruent) provided nearly identical results and therefore images are shown for paired t-tests due to their increased power. Significance for paired t-test was defined at a whole brain voxel wise threshold of $p < 0.01$ and an additional FSL's flame 1 cluster correction equivalent to $p < 0.05$ (Minimum 281 voxels). In addition, average percent signal change was extracted using the featurquery tool of FSL's software library and converting PE/COPE values to % for a priori anatomical ROIs. Anatomical bilateral masks, for the nucleus accumbens, thalamus, amygdala, hippocampus, superior frontal gyrus, middle frontal gyrus and anterior cingulate

cortex based on the Harvard Oxford cortical and subcortical atlases, were used for extraction of percent signal change for second level analyses in SPSS. These regions were selected based on previous research suggesting their potential relationship with stress (Lupien et al., 2009; Ulrich-Lai and Herman, 2009). Specifically, we tested the hypothesis that the twins exposed to greater stress have lower activation in lateral PFC regions and greater activation in the basal ganglia and limbic regions compared to their control co-twins without severe events. For the incongruent minus congruent contrasts, percent signal change was extracted for dlPFC and precentral gyrus ROI masks created from the significant I-C difference for the control co-twins (voxel wise threshold $p < 0.01$ and cluster corrected at $p < 0.05$). Percent signal change was also extracted for the anatomical amygdala MNI mask since the control co-twins did not show significant differences in the amygdala. Analyses comparing Congruent - Fixation and Incongruent - Fixation as well as analyses split by emotional blocks (Angry, Happy, Sad, and Neutral) are provided in the supplements (Supplemental Figs. S1 and S2). Analyses with depression and adulthood stress as covariates are also shown in the supplements (Supplemental Fig. S4).

4. Results

4.1. Behavioral

Table 1 shows the count and range of stressful life events as measured by the CARI-Q and Life Event Checklist at the selection time point. Consistent with selection criteria, the nine discordant twin pairs used in fMRI group comparisons showed significant differences for count of severe stressful life events during adolescence (paired $t_{(8)} = 3.59$, Wilcoxon $p = .007$). Although selection was based primarily on adolescent events, count of events which occurred during childhood and adolescence were also significantly different (paired $t_{(8)} = 8.71$, Wilcoxon $p = 0.02$) while counts of life events in past year was not significantly different (paired $t_{(8)} = 0.82$, Wilcoxon $p = 0.61$). The twins with stress also reported a higher number of childhood and adolescent events assessed at a second time point after the neuroimaging session (childhood: paired $t_{(8)} = 2.46$, $p = .039$; adolescence: paired $t_{(8)} = 2.71$, $p = 0.027$), which was not significantly different for adulthood (paired $t_{(8)} = 1.41$, $p = 0.20$). See also the Supplemental Table S2 for additional data from the second-time point LSCI and the Perceived Stress Scale (for the last month). These results are consistent with the most significant differences in stress being before age 18 and no significant differences for the Perceived Stress Scale.

Table 2 shows behavioral data for the emotional word-face Stroop task (reaction time, accuracy, and timeouts) for the sample

Table 1
Descriptive Statistics for Monozygotic Twin Pairs Discordant for Severe Stressful Life Events (SLE).

Measures	Twins with Stress			Control Co-twins			Paired <i>t</i> -test	<i>p</i> -value	Wilcoxon <i>p</i> -value	Cohen's <i>d</i>	Effect size (<i>r</i>)
	Mean	SD	Range	Mean	SD	Range					
Count of Severe Stressful Life Events											
Childhood/Adolescence 0–17 yrs	4.22	1.64	2–7	0.44	0.73	0–2	8.71	<0.001***	0.024*	2.98	0.83
Adolescence 9–17 yrs	2.22	1.64	0–5	0.11	0.33	0–1	3.59	0.007**	0.007**	1.78	0.67
Adulthood Past Year	6.56	4.04	1–13	5.11	2.80	1–9	0.82	0.44	0.611	0.42	0.20

Note. Significant results in bold. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, † $p < 0.10$, $n = 9$ twin pairs.

Table 2
Behavioral Performance during the Emotional Word-Face Stroop Task.

Measures	Mean	SD	Range	Mean	SD	Range	Paired <i>t</i> -test	
							Difference	<i>p</i> -value
	Congruent			Incongruent				
Reaction Time (ms)	729.36	62.65	664–875.1	752.68	58.09	672.7–882	17.944	<0.003
Accuracy %	0.90	0.09	0.69–1	0.89	0.08	0.71–0.98	0.006	0.538
Timeouts %	0.04	0.05	0–0.2	0.06	0.05	0.01–0.21	0.027	<0.001
	Twins with Stress			Control Co-Twins				
Congruent RT (ms)	734.66	70.64	686.9–875.1	724.05	58.72	664–837.8	10.404	0.786
Incongruent RT (ms)	757.89	65.37	698.8–882	747.46	54.55	672.7–840.3	13.464	0.714
I-C RT (ms)	23.23	14.78	6.93–51.19	23.41	16.47	2.48–45.22	3.061	0.718
I-C/C RT (ms)	0.03	0.02	0.008–0.073	0.03	0.02	0.003–0.061	0.003	0.755
Congruent Accuracy %	0.90	0.09	0.75–1	0.90	0.10	0.69–0.97	0.018	0.525
Incongruent Accuracy %	0.89	0.08	0.75–0.98	0.89	0.09	0.71–0.97	0.017	0.444
I-C ACC	–0.01	0.02	–0.03–0.03	–0.01	0.05	–0.09–0.06	0.001	0.967
I-C/C ACC	0.00	0.03	–0.03–0.04	0.00	0.05	–0.1–0.07	0.000	0.989
Congruent Timeouts %	0.05	0.07	0.00–0.20	0.04	0.04	0.00–0.12	0.005	0.643
Incongruent Timeouts %	0.06	0.07	0.009–0.21	0.05	0.04	0.01–0.13	0.009	0.702

Note. Significant results in bold. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, † $p < 0.10$, $n = 7$ twin pairs.

overall, as well as for comparisons between the twins exposed to severe stress and their control co-twins with lower stress. Consistent with interference effects, for the group overall, reaction time was significantly longer for incongruent trials compared to congruent trials ($t_{(13)} = 3.70$, $p < 0.001$), and a greater number of timeouts (>1500 ms) was found ($t_{(13)} = 4.39$, $p < 0.001$); however, accuracy was not significantly different between congruency ($t_{(13)} = -0.63$, $p = 0.54$). There were no significant differences between the twins with stress and their control co-twins for group and paired *t*-tests of reaction time, accuracy, and timeouts. Difference scores for incongruent minus congruent trials (I-C) as well as (I-C)/C, which accounts for baseline reaction time, were also not significantly different between the twins with stress and their control co-twins.

4.2. Task - fixation (goal maintenance)

4.2.1. Group-level activation patterns for task compared to fixation

Functional MRI BOLD signal (above a whole brain voxel wise threshold of $p < 0.01$ and a cluster correction $p < 0.05$) for overall goal maintenance during the emotional word-face Stroop task compared to fixation is shown in Fig. 2. Group level activation for the control co-twins with low stress is shown in Fig. 2a and for the twins with higher stress in Fig. 2b. The control co-twins showed frontoparietal activation typical of Stroop task performance. Although the twins with stress also showed frontoparietal activation, it is reduced in extent. Most notably, the twins with stress showed activation above threshold in limbic and basal ganglia regions while their control co-twins do not show this activation, and instead show deactivation of medial and frontal orbital regions compared to fixation.

4.2.2. Paired *T*-tests (Twin with stress > control co-twin)

Fig. 3 shows significant differences in BOLD signal between twin pairs (paired *t*-test $p < 0.01$ and cluster correction $p < 0.05$). This

shows that twins with stress compared to their control co-twins showed significantly greater activation in prefrontal and limbic regions including the ventrolateral prefrontal cortex (vlPFC), dorsal and pregenual anterior cingulate cortex (ACC) into medial prefrontal regions, basal ganglia, hippocampal and parahippocampal regions. Table 3 shows the peak coordinates and cluster sizes for significant activation differences between the twins with stress and their control co-twins. This pattern of activation is consistent with the pattern at $p < 0.005$ with no cluster correction (Supplemental Fig. S3). The vlPFC, medial PFC, and hippocampus remained significant even after controlling for depression, and adulthood stress (paired *t*-test $p < 0.01$ and cluster correction $p < 0.05$). The anterior cingulate, however, was associated with higher depression symptom counts and the basal ganglia regions did not pass cluster significance after controlling for depression but were significant at a voxel wise threshold of $p < 0.005$ without cluster correction (see Supplemental Fig. S4).

To further illustrate the relationship between twins in percent signal change in these regions and show that one or two people are not driving the effect, group dot plots of a priori ROIs are shown in Fig. 4 and descriptive statistics are shown in Table 4. Paired *t*-test were significant for percent signal change for the left and right nucleus accumbens, thalamus, hippocampi, right amygdala ($p < 0.05$) and trending for the anterior cingulate cortex. Additional a priori regions that were tested but were not significantly different were the left amygdala, middle frontal gyrus (MFG), and superior frontal gyrus (SFG).

4.3. Incongruent - congruent

4.3.1. Group-level activation for incongruent compared to congruent

Fig. 5a and Table 5 shows group level activation (whole brain voxel wise $p < 0.01$ and cluster correction $p < 0.05$) for the control co-twins in the dorsolateral PFC and pre and postcentral gyrus for

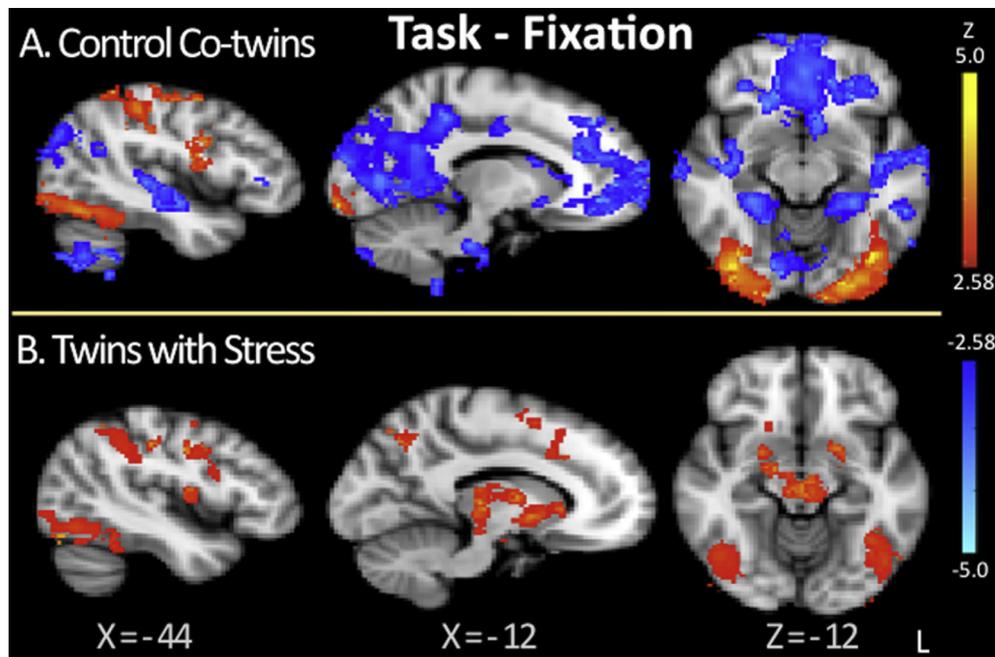


Fig. 2. Task - Fixation. The control co-twins showed activation (red-yellow) in the dlPFC and parietal cortex and deactivation (blue-light blue) of the medial and orbital frontal regions. Their genetically identical twin exposed to severe stress during development showed additional activation in the basal ganglia, hippocampus and amygdala throughout the task compared to fixation (Voxel wise threshold $p < 0.01$ and cluster corrected $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

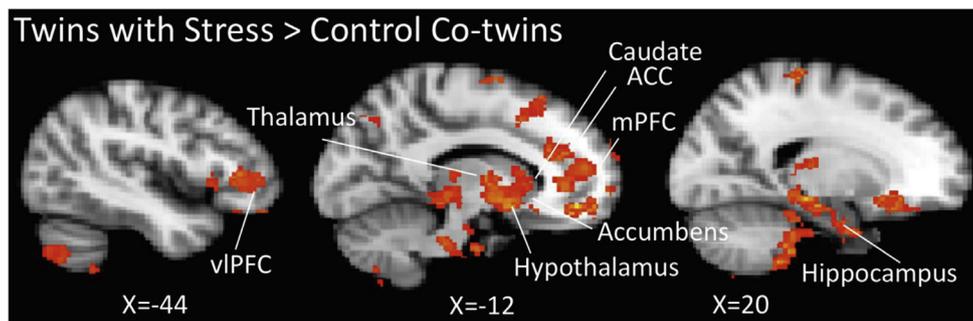


Fig. 3. ST > CT Task - Fixation. Twins exposed to stress showed greater activation in the ventrolateral prefrontal cortex (vIPFC), medial prefrontal cortex (mPFC), limbic and basal ganglia regions compared to their control co-twins who showed deactivation during the task (Voxel wise threshold $p < 0.01$ and cluster corrected $p < 0.05$).

Table 3

Activation during the Emotional Word-Face Stroop Task for Twins with Stress greater than Control Co-twins.

Brain Regions	Max Z	voxels	MNI coordinates		
			x	y	z
Frontal Medial into Basal Ganglia and Limbic regions	4.71	8814	-12	46	-12
Superior Frontal Gyrus	4.63	1058	-4	10	72
Superior Parietal and Occipital Cortex	4.45	495	-14	-74	44
Left Cerebellum	4.07	422	-40	-66	-48
Medial Cerebellum	4.58	360	10	-72	-50
Anterior Cerebellum into Brainstem	4.24	330	-24	-44	-54

Note. No significant clusters for Control Co-twins > Twin with Stress. All results pass voxel wise threshold $p < 0.01$ and whole brain cluster correction $p < 0.05$.

the incongruent minus congruent (I-C) contrast (these regions were used to create ROI masks for extracted percent signal change). Only the control co-twins showed significant frontoparietal activation greater for the incongruent blocks compared to the congruent blocks (I-C). Twins with stress did not show significant differences between the incongruent minus congruent contrast and is therefore not shown.

4.3.2. Paired T-tests (Twin with stress > control co-twin)

Fig. 5b shows paired t -tests were consistent with group level distinctions suggesting that there were differences between the control co-twins and the twins with stress in the dlPFC, precentral gyrus, lateral occipital cortex, and amygdala for the incongruent minus congruent contrast. These regions were significantly different at a voxel wise threshold of $p < 0.01$, but did not reach a

Table 4
Descriptive Statistics for Extracted Percent Signal Change during the Emotional Word Stroop Task > Fixation.

Measures	Twins with stress			Control co-twins			Paired <i>t</i> -test	p-value	
	Mean	SD	Range	Mean	SD	Range			
Left									
Accumbens	0.13	0.13	-0.06–0.33	-0.08	0.12	-0.32–0.06	3.33	0.010	*
Thalamus	0.11	0.12	-0.13–0.27	-0.07	0.17	-0.42–0.07	2.44	0.040	*
Amygdala	0.10	0.14	-0.14–0.32	-0.02	0.22	-0.37–0.40	1.80	0.110	
Hippocampus	0.04	-0.11	-0.20–0.25	-0.11	0.13	-0.32–0.001	3.47	0.009	**
Right									
Accumbens	0.11	0.15	-12–0.35	-0.09	0.13	-0.35–0.06	2.68	0.028	*
Thalamus	0.09	0.12	-0.16–0.23	-0.07	0.17	-0.39–0.10	2.27	0.053	†
Amygdala	0.13	0.16	-0.14–0.36	-0.04	0.18	-0.33–0.29	3.66	0.006	**
Hippocampus	0.06	0.16	-0.23–0.24	-0.10	0.10	-0.24–0.008	3.93	0.004	**
Prefrontal Cortex									
ACC	0.04	0.15	-0.17–0.37	-0.11	0.13	-0.35–0.05	2.10	0.069	†
SFG	0.11	0.32	-0.26–0.81	-0.14	0.33	-0.72–0.52	1.54	0.162	
MFG	0.11	0.25	-0.23–0.62	-0.05	0.24	-0.52–0.36	1.23	0.253	

Note. Significant results in bold. **p* < .05, ***p* < .01, ****p* < .001, †*p* < 0.10, n = 9 twin pairs.

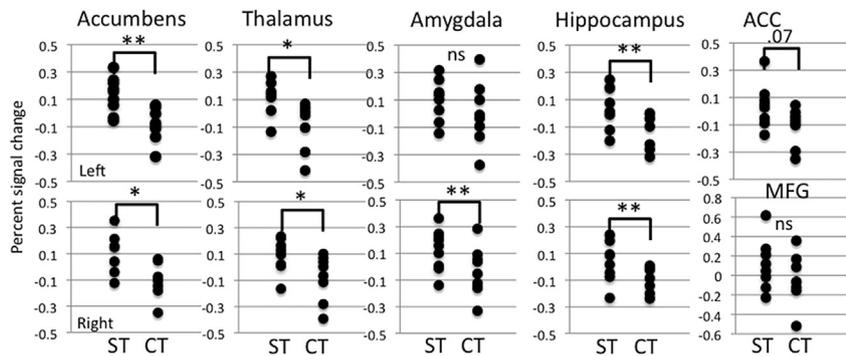


Fig. 4. Percent Signal Change for a priori ROIs. Twins with stress (ST) showed greater percent signal change in limbic and basal ganglia regions compared to their control co-twins (CT). Paired *t*-test; ***p* < 0.01, **p* < 0.05.

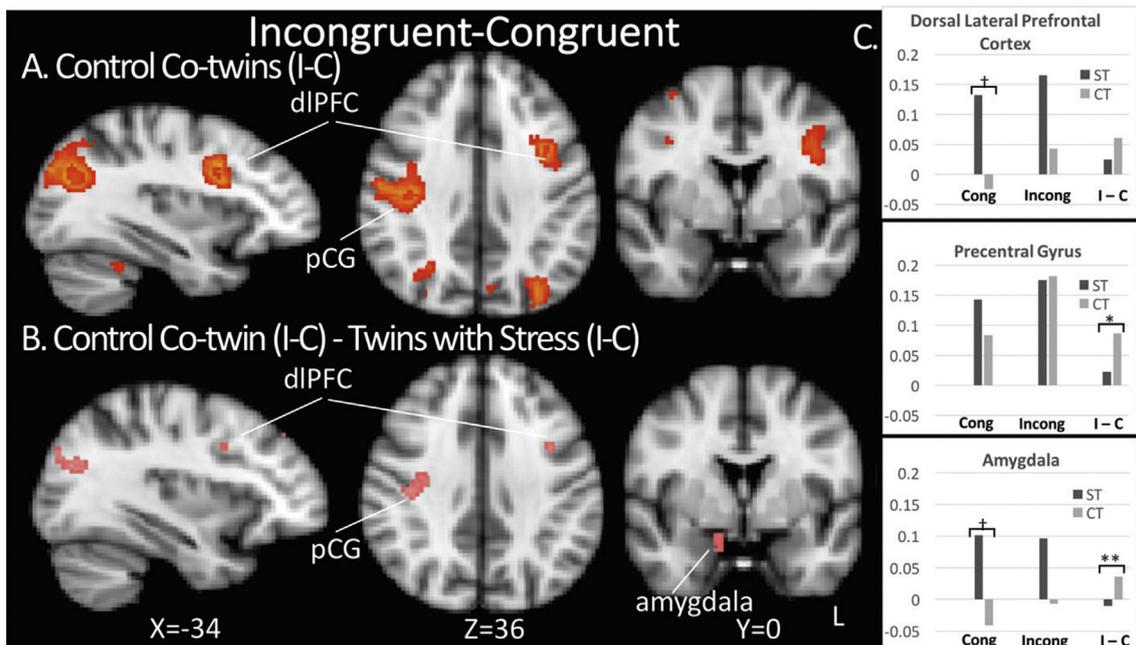


Fig. 5. Incongruent-Congruent (I-C). A. Activation was greater for incongruent compared to congruent trials (I-C) for the control co-twins only (Voxel wise *p* < 0.01 and *p* < 0.05 cluster correction). There were no significant differences for the twins with stress I-C (not shown). The difference between Incongruent and congruent for control co-twins compared to their twins with stress is shown in Panel B (Voxel wise *p* < 0.01, no cluster correction). Panel C. shows percent signal change extracted from regions of interest (Post hoc *t*-tests ***p* < 0.01, **p* < 0.05, †*p* < 0.10. Only the amygdala difference passed multiple test corrections). dlPFC, dorsolateral prefrontal cortex; pCG, postcentral gyrus.

Table 5
Incongruent > Congruent for Twins with Stress and Control Co-twins.

Brain Regions	BA	Max Z	voxels	MNI coordinates		
				x	y	z
<i>Twins with Stress</i>						
No significant clusters						
<i>Control Co-Twins</i>						
Superior Lateral Occipital cortex and precuneus	BA 19	4.12	2276	–16	–70	56
Precentral and Postcentral Gyrus	BA 1, 2, 3, 4	4.58	1521	40	–16	40
Middle Frontal Gyrus	BA 46	4.18	754	–34	8	34
Posterior lobe of Cerebellum		4.01	630	12	–84	–44
Occipitotemporal Fusiform Cortex	BA 37	3.91	378	–26	–52	–22

Note. No significant clusters for Control Co-twins > Twin with Stress. All results pass whole brain voxel wise threshold $p < 0.01$ and cluster correction $p < 0.05$.

cluster correction of $p < 0.05$. Panel C and supplementary results (Fig. S1) further parse activation of each block type (congruent and incongruent) compared to fixation. Panel C shows the percent signal change extracted from ROI masks created from the difference between I-C in the control co-twins as well as the MNI anatomical amygdala mask. These results suggest that the control co-twins showed greater activation in the dlPFC and precentral gyrus during the incongruent condition compared to the congruent condition while the twins with stress showed only a small non significant difference. In contrast, the twins with stress showed greater activation in the amygdala and hippocampal regions on incongruent trials and congruent trials compared to their control co-twins. The results in panel B did not pass cluster correction and only the amygdala passed post hoc, multiple test corrections in panel C. Therefore, these results should be considered preliminary.

In summary, limbic and basal ganglia regions as well as ventrolateral and medial PFC regions showed greater BOLD signal in the twins exposed to severe stress compared to their control co-twins throughout the task. Control co-twins showed greater activation in the MFG, precentral gyrus, and lateral occipital cortex during incongruent trials compared to congruent trials, while the twins with stress did not show these differences. Overall, these results were significant for all emotional blocks suggesting that the emotion of the face was not driving the medial and ventrolateral differences between twin pairs (See [Supplemental material, Fig. S2](#)).

5. Discussion

This study showed significant differences in prefrontal and limbic system activation during an emotional word-face Stroop task in a small and rare sample of monozygotic twins who were discordant in exposure to severe stress during development. Specifically, the twins that experienced multiple severe stressful life events showed greater activation of the medial and ventrolateral PFC, basal ganglia, amygdala and hippocampal regions throughout the task compared to their control co-twins. The control co-twins with little to no exposure to severe stress showed more typical frontoparietal activation patterns consistent with previous research on Stroop performance (Banich et al., 2000; Compton et al., 2003). These findings are consistent with previous research highlighting functional and structural differences in the limbic system and PFC associated with exposure to stress (Arnsten, 2009; Lupien et al., 2009; McEwen et al., 2015). Here we extend prior research by demonstrating, for the first time, that even in twin pairs who were genetically identical and reared together, exposure to stress during development may lead to differential recruitment of limbic and executive control regions during a challenging executive function task. This study highlights how exposure to stress before age 18, may lead to a neural system that is more reactive to emotional and potentially stressful stimuli, thereby allocating resources to brain

regions not typically recruited to solve the task.

5.1. Maintaining the goal

The emotional word-face Stroop task used in this study was designed to assess the ability to maintain the task goal of responding to words presented on emotionally distracting faces. Therefore, this task is able to tap into several processes that are likely to be associated with stress and stress-related EF deficits. Throughout the task, the control co-twins showed recruitment of regions within the frontoparietal network, consistent with maintenance of task goals and overcoming distraction from emotional stimuli (Compton et al., 2003; Jordan et al., 2013). This network typically shows greater activation during performance on EF tasks and is considered essential to goal maintenance. In contrast, the twins with higher stress recruited regions of the frontoparietal control network to a slightly lesser degree, and significantly recruited more ventral and medial regions of the prefrontal cortex as well as the basal ganglia and limbic system compared to fixation.

We speculate that individuals with stress may have been simultaneously updating the task goal information related to the word and the task irrelevant information available in the facial expressions, thereby leading to increases in activation of the basal ganglia, medial and ventral PFC, and limbic system, in addition to the frontoparietal activation necessary for the Stroop task. The basal ganglia and midbrain more generally are considered major players in updating of working memory as well as more automatic behavior, stress reactivity and reward (Alexander and Crutcher, 1990; D'Ardenne and Eshel, 2012; Lombo and Giménez-Amaya, 2014; Schwabe and Wolf, 2011). In other words, one pathway to solve the task is to maintain only the goal at hand, while an alternative route, albeit costlier in terms of resources, is to maintain the goal of the word as well as process the emotion facial information while updating back and forth. Although preliminary, the group difference found in the anterior cingulate was associated with symptoms counts of depression while the vlPFC, hippocampus and medial PFC remained cluster significant. Additional activation specific to depression was observed in the posterior cingulate as well as the insula, medial parietal and dorsal lateral frontal (dlPFC) regions. While adulthood stress did not impact the differences found between the twins, additional effects were observed in the posterior cingulate, precentral and occipital regions. Thus, the twins with stress seemed to be allocating additional resources that were essentially accumulating with stress exposure and symptomatology.

This neuroimaging data is consistent with work in a large normally distributed sample, which showed that stressful life events, whether positively or negatively perceived, typical or severe, were associated with lower scores on a common EF latent factor (Godinez, et al., Unpublished results). After accounting for the negative relationship between stress and this common EF latent factor, which is thought of as goal maintenance (Miyake and Friedman,

2012), scores on the updating-specific factor seemed to increase for positive and moderate stress but showed a wide range of variability in terms of the relationship with severe stress. As researchers better parse the differences amongst EF specific components, we will better understand the relationship between stress, goal-directed processing, updating between multiple sources of information, mental health symptomatology and brain development. It will also be important to assess the interaction of more top-down EF processes compared to more bottom-up attentional biases as it was clear that the twins with stress were utilizing neural resources associated with processing salient or potentially emotional stimuli. This social/emotional information was task irrelevant but may be life relevant to individuals who experience stress.

5.2. Flexibility

The incongruent minus congruent contrast is thought to measure the ability to increase processing when the task irrelevant information is conflicting or incongruent. During the incongruent blocks compared to congruent blocks (I-C), the frontoparietal regions typically show greater activation during the Stroop task (Banich et al., 2000). This I-C frontoparietal activation was found in the control co-twins such that the control twins showed greater activation in the dlPFC and pre and postcentral gyrus, which survived cluster correction. There were no significant differences for the twins with stress, suggesting that the twins with exposure to severe stress were recruiting similar brain regions during both incongruent blocks and congruent blocks. In general, the twins with stress showed similar activation patterns throughout the task while their control co-twins showed activation patterns that varied depending on the trial type as shown by I-C and emotional block comparison; however, differences between twin pairs for the I-C contrast did not pass cluster corrections and should be replicated in a larger sample (See supplemental data). None the less, the results suggests that one component of goal-directed cognition that may be related to stress exposure is the ability to flexibly recruit resources according to the task demand.

Both the twins with stress and the control co-twins showed significantly slower reaction time and a greater number of timeouts for incongruent trials compared to congruent trials, which is consistent with interference effect found in Stroop-like tasks. Contrary to our hypotheses, however, twins with stress did not show significant differences in behavioral performance compared to their control co-twins. This finding is consistent with other discordant MZ studies on PTSD (Shin et al., 2011) and depression (Wolfensberger et al., 2008) that showed differences in brain activation patterns while behavioral performance was not significantly different between the twins. It is possible that with increased severity, increased task demand and a larger sample, we would be able to detect the behavioral performance differences found in larger datasets. Alternatively, activation patterns may reflect a compensation effect which allowed the twins to behave similarly in performance. Overall, these findings highlight the sensitivity of the discordant fMRI design. Although this study does not have the power to assess the temporal dynamics of the task, in general the neuroimaging and behavioral data was more consistent with incongruent interference effects in both twins, while the twins with stress seemed to maintain this level of attention and allocation of resources throughout the task and therefore more generally (Britton et al., 2006; Carretié, 2014; Pessoa et al., 2002).

5.3. When does a challenge become a stressor?

If we extrapolate to everyday life, individuals who were exposed to severe stress during development may be using more resources

compared to those not exposed to stress to solve the same problems. This adaptation to stress has the potential to feel more demanding during challenging tasks as well as lead to avoidant strategies to conserve resources, or consumption behaviors to restore resources to their brain and body. Stressful life events have indeed been associated with a wide range of disorders including depression, anxiety, conduct disorder, substance use, and eating disorders, thus implicating stress in both internalizing and externalizing disorders (Andersen and Teicher, 2008; Crowley et al., 2003; Duggal et al., 2000; Felitti et al., 1998; Kendler and Gardner, 2010; Lucassen et al., 2014; Williamson et al., 2003). The data presented in this study are very consistent with models that describe how experiences of stress during development are predictive of functional changes in the hippocampus, amygdala, and prefrontal cortex (McEwen et al., 2015; Sousa, 2016). Exposure to severe events was also a moderate predictor of mental health symptoms, which showed additional differences consistent with recent reviews (Hall et al., 2015; Lucassen et al., 2014). Given these individuals were children and adolescents during the time of their stressful experiences, the results underscore the increased social responsibility to provide safe environments and the potential for interventions that focus on alleviating the effects of stress (Hariri and Holmes, 2015).

Although this task was not designed to be a stressor, it is particularly interesting that the twins with stress showed increased activation in regions that are typically activated during a stress response. This raises the speculation, to be tested in future studies, that MZ twins with higher stress exposure during development elicited a greater stress response to solve the same task as their control co-twins. If exposure to two or more severe stressful life events during development can differentiate whether the same event is experienced as challenging or stressful in the future, stress may accumulate over a lifetime. This interpretation is consistent with many theories of stress including allostatic load, stress accumulation, neuropathy and neuromatrix of stress, and even the stress generation hypothesis (Juster and McEwen, 2010; Lucassen et al., 2014; McEwen, 2004; Sousa, 2016), which all suggest that early time points of stress are associated with the increased likelihood of reporting more stressful events at later time points, thereby contributing to the negative cycle of stress. Evidence from animal studies, suggests that one underlying neurobiological mechanism whereby stress has negative effects on brain structure is dendritic atrophy and spine density reduction in the PFC and hippocampus, and increased connectivity and excitability of the amygdala and basal ganglia (Arnsten, 2009; Pruessner et al., 2009; Sinclair et al., 2010; Ulrich-Lai and Herman, 2009), which is also associated with epigenetic changes (Griffiths and Hunter, 2014; McEwen et al., 2015; Sousa, 2016). Together, this research suggests that interventions focused on alleviating stress during childhood and adolescent development (Hariri and Holmes, 2015) may be potential avenues for interventions that translate across genetic background.

5.4. Limitations

Without debate, a small sample selected at random is not likely to be representative of the population. However, the present sample was *not* randomly selected, but rather selected from a larger sample of over 290 MZ twin pairs with only severe stressful life event data. Several recent articles have endorsed discordant twin designs and the power of the MZ discordant designs in particular (Blokland et al., 2012; van Dongen et al., 2012; Zwiijnenburg et al., 2010). By utilizing paired t-tests and scatter plots from individual data, we feel confident that this effect is not being driven by one or two twin pairs. Furthermore, this research is consistent with the

discordant twin research on PTSD (Gilbertson et al., 2002; Shin et al., 2011) and depression (de Geus et al., 2007) that shows similar regional differences, thereby adding to a converging body of evidence that demonstrate differences in limbic and prefrontal brain activation despite common genetics and shared familial environment. Although not part of our a priori analysis plan, twin pairs discordant for stress also showed a trend for differences in depression symptoms counts and total count of anxiety, depression, and antisocial symptoms (see [supplemental data](#)); However, this sample is best described as stress discordant since over half of the pairs were concordant for 0 or 1 of each type of mental health symptom. Despite the small sample size and limited access to mental and physical health symptoms (PTSD, medical issues, etc.), adding depression symptoms and adulthood stress as covariates resulted in similar activation patterns. The exception was the anterior cingulate, which was associated with depression symptoms and the basal ganglia, which no longer reached cluster significance but was significant at $p < 0.005$. Future research will be important to clarify the role of stress during development and mental health symptoms, which may underlie some of these differences.

Although we originally selected twin pairs discordant on stress during adolescence, we found that those who experienced stress during this period were also more likely to have an onset or additional stressful experiences during childhood. Therefore, we refer to our sample as having stress during development and cannot rule out the potential effects of stress earlier in life. Selection was, however, focused on events that had an onset in the later years of childhood and that continued into adolescence. In general, MZ twins may be more likely to have discordant differences in the later years and concordant experiences prenatally and earlier in childhood. Although not conclusively, this research suggests that it is important to examine other developmental time points in addition to early life events as stress during preadolescence and adolescence may be more consistent with the effects demonstrated in this paper. Additionally, it is important to consider how stress earlier in development influences how stress is experienced in adulthood, as we had limited power to detect smaller effects. Future research will examine developmental time points more systematically as well as developmental influences related to structural alterations.

6. Conclusion

Overall this research shows that despite identical genetics and shared familial experiences (i.e., socio-economic factors, prenatal influences, shared family and schooling), two or more severe stressful life events during development can differentiate brain activation patterns during an emotional word-face Stroop task in young adulthood. In general, twins exposed to severe stress showed greater activation of the ventral and medial regions of the PFC, basal ganglia and limbic system compared to their control co-twins, suggesting that twins exposed to severe stress were employing more resources to solve the same task and potentially experiencing a heightened level of stress during the task. Although this research is not the first to show the relationship between stress and emotional and cognitive control, it does provide a clear example found even in reared together genetically identical twins.

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Conflicts of interest

None of the authors have any conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jynstr.2016.10.002>.

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