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Early-life stress exposure associated with altered prefrontal resting-state fMRI connectivity in young children

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ABSTRACT

Early-life stress (ELS) exposure is associated with adverse outcomes across the lifespan. We examined the relation of ELS exposure to resting-state fMRI in children ages 4–7 years. ELS in the first years of life, but not concurrent, was associated with higher regional homogeneity of resting-state fMRI in the left lateral frontal cortex. Resting-state fMRI functional connectivity analyses showed that the region of left lateral frontal cortex demonstrating heightened regional homogeneity associated with ELS was negatively correlated with right temporal/parahippocampal areas. Moreover, higher regional homogeneity in the left lateral frontal cortex and its negative coupling with the right middle temporal/parahippocampal areas were associated with poorer performance on a reversal-learning task performed outside the scanner. Association of ELS exposure with regional homogeneity was independent of other early adversities. These findings suggest that ELS may influence the development of cognitive control in the lateral prefrontal cortex and its interactions with temporal cortex.

1. Introduction

Early-life stress (ELS) has been used to refer to a host of negative experiences in childhood including exposure to stressors such as acute events, socio-economic inequalities-related stressors, and perceived stress. ELS is associated with adverse outcomes across the lifespan (Lupien et al., 2009). Retrospective studies link extreme adverse childhood experiences (e.g. maltreatment) to a range of mental health problems. More recently, multiple studies demonstrated links between exposure to mild chronic stressors and disinhibited behavior as manifest in early childhood externalizing problems (Glover, 2011). These findings suggest that ELS' link to behavioral disinhibition can be detected early in life, but mechanisms are poorly understood. Negative effects of ELS could derive from deleterious effects of stress on neurodevelopment. However, our understanding of the relation between ELS and brain maturation in early childhood years remains limited. Here we examine the association of ELS during early childhood to resting-state fMRI at preschool age.

Rodent and nonhuman primate models and retrospective studies in human adults have revealed deleterious effects of ELS on neural function in brain networks important for memory, arousal, cognitive control, and reward processing, and other difficulties in associated cognitive functions (Lupien et al., 2009; Teicher et al., 2003; Woon and Hedges, 2008). A growing body of literature on the effects of severe adverse childhood experiences, such as maltreatment or neglect, in children reveals relations to physiological stress responses as well as structural and functional brain differences (Lupien et al., 2009; Mackey et al., 2012; Pechtel and Pizzagalli, 2011). Institutionalization and maltreatment history are associated with differences in amygdala, hippocampal, cingulate and prefrontal gray matter volume and white matter structure, and altered activation and connectivity in frontal areas, amygdala and

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striatal areas compared to controls (Burghy et al., 2012; Chugani et al., 2001; De Bellis et al., 2002; Eluvathingal et al., 2006; Mehta et al., 2009; Hanson et al., 2010; Mueller et al., 2010; Tottenham and Sheridan, 2009). The findings of amygdala and hippocampal differences in the literature are less consistent than differences in prefrontal cortex (which contributes to cognitive control), with some studies revealing no differences in the former set of regions as a function of early-life extreme stress (Kim et al., 2013; Mackey et al., 2012; Pechtel and Pizzagalli, 2011; Woon and Hedges, 2008). Thus, prefrontal cortex might be especially sensitive to stress as compared to amygdala and hippocampus (Mackey et al., 2012).

Exposure to extreme stressors, such as institutionalization, is rare. Social inequalities-related stressors such as poverty have been recently used as a rough, distal indicator of more common, milder psychosocial stress exposure. Low childhood socioeconomic status (SES) is associated with structural and brain differences in areas central to cognitive control and verbal processing (Hackman and Farah, 2009). Directly focusing psychosocial stress exposure, Hanson and colleagues (2012) reported associations between cumulative life stress and reduced prefrontal cortex volume beginning in early childhood. Buss and colleagues (Buss et al., 2010, 2011) showed that exposure to normative types of maternal perceived stress and distress (pregnancy-specific anxiety) predicts brain and executive function alterations at school ages.

While providing compelling evidence for associations of ELS with brain and cognitive development, previous studies primarily focused on older school-aged children, compared different groups of children exposed to different forms of ELS, used cross-sectional designs, and mainly used structural brain measures. The first goal of this study is to demonstrate the relations between measures of psychosocial stress across early childhood and young children's resting-state fMRI in a demographically diverse sample of preschool-children. We use an interview-based measure of maternal perceived stress as our measure of ELS. We also examine whether associations with ELS are independent of other stress indicators, such as poverty, acute life events, and violence exposure. Perceived stress is a good indicator of individual differences in impact of stress, because the same stressor may have differential impact biologically and psychologically depending on how stress is appraised and experienced (Graignic-Phillippe et al., 2014). The second goal of this study is to examine effects of timing of exposure i.e., first years of life vs. concurrent preschool-age stress on neural outcomes on resting-state fMRI within the same group of children.

The current study used resting-state fMRI (rs-fMRI), which is considered to reflect spontaneous connectivity in the brain during rest (Long et al., 2008). Studies with adults report atypical resting-state fMRI patterns in clinical populations suffering from mental health disorders that are associated with ELS, such as anxiety disorders and populations suffering from post-traumatic stress disorder (Sripada et al., 2012). rs-fMRI might reveal information about stress-related differences in the brain that exist at baseline in the absence of any stress-related stimuli or task demands. To our knowledge, no studies have examined how psychosocial stress exposure in early life is linked to rs-fMRI in young children. As our measure of rs-fMRI, we used regional homogeneity (ReHo) analysis that measures temporal similarity of voxels within a cluster and is considered to reflect intra-regional synchronization in a network (Song et al., 2011). We followed these ReHo analyses with network-based resting-state functional connectivity (rs-FC) analyses to identify associations with ELS for other regions that are functionally connected with those identified by the ReHo analysis. Given the past literature on ELS' relations to brain differences in prefrontal regions, we expected ELS to associate most strongly to ReHo and fMRI connectivity in the prefrontal regions.

Given the previous literature highlighting relations between ELS and prefrontal functioning, we also examined how differences in ReHo and rs-FC relate to children's performance on the behavioral cognitive-control task, which is associated with prefrontal functioning (e.g. Hackman and Farah, 2009; Mackey et al., 2012). Cognitive control refers to the ability to flexibly coordinate thoughts and behavior to accomplish goals. One of its basic components is the ability to adapt to shifting task demands in a flexible manner, as opposed to remaining rigid. Such task switching is supported by a wide fronto-parietal network in the brain, and resting connectivity in this network is linked to behavioral measures of cognitive control (Kim et al., 2012; Seeley et al., 2007). In the current study, we used a developmentally engaging task to measure reversal-learning (Dias et al., 1996; Finger et al., 2008; Pryce et al., 2005). On the basis of the past literature suggesting that ELS specifically associates with structural and functional brain differences in prefrontal regions, we expected ELS-related neural differences to associate with children's performance on the behavioral cognitive-control task (e.g. Hackman and Farah, 2009; Mackey et al., 2012).

Overall, the current study aims to demonstrate the relations between exposure to ELS and resting-state fMRI in a demographically diverse sample of young children. We aim to add to the existing literature by (1) establishing specific associations between ELS and neural differences relative to other indicators of stress such as poverty, acute life events, and exposure to violence, (2) examining whether the associations with ELS varies depending on its timing, by comparing ELS in the first years of life to Concurrent Stress at preschool age, and (3) examining whether associations between prefrontal regions and ELS are related to differences in cognitive control.

2. Materials and methods

2.1. Participants

Participants included an intensive sub-sample of a socioeconomically diverse community-based cohort participating in a larger study of preschool psychopathology (see Wakschlag et al, 2015). At the time of the intensive assessment, the mean age of this sample was 5.1 years-old (range 4–6.3 years). The intensive visit consisted of two, several-hour assessments typically conducted within a few weeks of each other. The ELS interview was administered in the second visit. After completion of the second visit, those children eligible for the resting-state sub-study were invited to participate.

Children were selected for the current study based being high versus low on a developmentally-sensitive measure of Temper Loss on the Multidimensional Assessment Profile of Disruptive Behavior (MAP-DB) (see below for grouping criterion) (Wakschlag et al., 2014). Selection into the resting-state sub-study was also based on prior performance during an ERP assessment administered to participants who attended the second day of the baseline visit. Children were administered three ERP tasks. If a child was able to complete all three EEG tasks, they were ranked as a "good" candidate for the fMRI study. If a child was able to complete two out of the three tasks, they were ranked as "moderate" candidates for the fMRI study. Children who completed one or less of the three tasks were ranked as poor candidates for the fMRI study. Children who had missing data or did not complete the ERP tasks were ranked as "unsure" candidates for the fMRI study. Participants who were ranked as "good" candidates were contacted first, followed by the "moderate" candidates, followed by the "poor" and "unsure" candidates.[1]

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[1] A total of 80 families were invited into the resting-state study. Four children were ineligible due to claustrophobia or epilepsy. Of the remaining 76 families, 7 declined participation. Of the remaining 69 families, 52 participated, 4 did not show

Of the fifty-two subjects who completed the ERP studies 15 were recruited from the "good" candidates, 24 from the "moderate" candidates, 6 from the "poor" candidates, 7 from the "unsure" candidates. Forty-two children completed full resting-state scans. One child was identified as ineligible for the current paper due to expressive and receptive language delays. Of the remaining 41 children, 13 were excluded because of excessive movement in the scanner (maximum head movement more of more than 3 mm or 3° in any direction), or poor whole-brain coverage (i.e. insufficient coverage of the superior parietal or inferior temporal lobe). Thus, 28 (16 girls) children are included in the current analyses.

Study eligibility required that all children were free of a serious developmental disability or delay, e.g. autism. Children's ages ranged from 4.5 to 7.5 years at the time of the rs-fMRI scan ($M = 6.1$ years, $SD = 0.7$ years). The time between the ELS assessment and participation in the resting-state study varied from .2 to 22.8 months ($M = 11$ months, $SD = 6.2$ months). The sample was diverse (54% African-American, 43% Caucasian, 1% biracial and 21% Hispanic). There was written assent for subjects over the age of six and verbal assent if the subject was under the age of six. Consent was given by the child's mother. All experimental procedures were approved by the Institutional Review Board at Northwestern University. Poverty status was determined via an income-to-needs ratio. Based on this 16 children were classified as poor.

Child assessment. Developmental and neurocognitive abilities were assessed during an intensive laboratory visit at baseline.

Nonverbal IQ. The *Differential Ability Scales*-Second Edition (DAS-II) (Elliott, 1983) Picture Similarities subscale provided a measure of developmental level.

Temper Loss. As part of the larger MAPS investigation of the neural substrates of early irritability, children were selected into the resting-state sub-study based on their *Temper Loss* scores on the MAP-DB (Wakschlag et al., 2014). Using population-based thresholds from an independent sample of 1488 preschoolers (Wakschlag et al., 2012), high Temper Loss was defined as at or above the 80th percentile and low Temper Loss was defined as below the 50th percentile. Using this criterion, 14 children were categorized as low and 14 high on Temper Loss.

Reversal-learning. Children's response reversal was assessed via a computerized developmentally sensitive "*Candy Game*" task in which children were presented with two sets of boxes (adapted from Crowley and Mayes, 2004). Children were presented intermittently with two sets of boxes. One box is designated as "winning," and one box is designated as "losing" in each set. The goal was to touch the "winning box" in each trial, and in return gain one piece of candy. If a "losing box" was selected, one piece of candy was taken away. The same boxes were the "winning" and "losing" boxes for the first part of the game (pre-switch). At a certain point the first set of boxes were changed and the "winning" box became the "losing" box and vise versa (post-switch). The experiment had 120 trials (60 trials per box set). Each set of boxes was reversed after 30 trials. Each trial was self-paced. The task was performed on a touch screen monitor. Participants were required to learn the rewarded stimulus by trial and error. Our measure of reversal-learning was post-switch minus pre-switch percent accuracy, indicating the decrement in children's performance due to the rule switch. Lower decrement in performance after the rule switch was considered to indicate better reversal-learning. Three of the 28 children had less than 50 trials and/or scored 50% or lower in the first half, and thus were excluded from analyses of reversal-learning.

Mother interviews. Mothers were interviewed at baseline to assess children's ELS and violence exposure. The ELS interview was conducted at the second baseline visit.

Early-life stress. Stress exposure was calculated based on information from an adaptation of the *Family Socialization Interview (FSI)*. The *FSI* is an open-ended, semi-structured interview about child developmental history, socialization, and family background, developed by Bates, Dodge and colleagues (Dodge et al., 1994). The *FSI* has been used with demographically diverse samples (Jaffee et al., 2004). ELS was assessed across two developmental periods: (1) *Exposure to Stress in Early Development*, i.e., from the child's birth through one year prior to baseline. The length of time covered by this developmental period varied since at baseline child were stratified by age across ages 3–5 (18% of early scores children covered the period through age 3, and 82% covered ages 4–5), (2) *Concurrent Exposure to Stress*, i.e., the past year to the time of the baseline assessment. For each of these periods, mothers were queried about any "changes or adjustments" that occurred in the family with an open-ended question followed by prompts, and then queried about specific events. This adapted version of the FSI queried 42 events (e.g. extended parent–child separation, death of a close family member, financial strain, medical problems, housing challenges). Mothers were asked to indicate how stressful each event was on a scale ranging from 0 (event did not occur) to 3 (occurred and very stressful). At the end of this reporting, mothers were asked to provide global rating of their perceptions of how stressful these events were for their family within each developmental period, on a scale from 1 (minimal stress) to 5 (severe stress). The primary variables used in analyses were mothers' ratings of global family stress. Total number of events reported within each period was also used in analyses as a control of acute life events.

Violence exposure. Mothers completed a modified 52-item version of the *Revised Conflict Tactics Scale* (CTS2, Straus et al., 1996), a questionnaire about physical aggression between mother and her partner during conflicts (e.g. slapping, grabbing, and using a knife or gun). We defined partner violence as presence of 1 or more physically aggressive tactics during partner conflicts since these events had low base-rate (<13% of sample). Mothers also completed the *Parent–Child Conflict Tactic Scales* (CTSPC, Straus et al., 1998), a 22-item questionnaire concerning tactics used during conflicts with her child, including physical and psychological aggression, and other tactics. The eight physical aggression items ranged from spanking to more severe behaviors (e.g., hitting the child with a fist). To ensure that physical aggression toward the child reflected more than spanking, parent–child violence exposure was defined as the presence of 2 or more physically aggressive tactics: 10 children were exposed and 17 were not. One (1) child did not have violence exposure data and thus was excluded from violence exposure analyses.

2.2. rs-fMRI procedure

The rs-fMRI protocol lasted ~1½ h. The first hour of the protocol included stillness training with a mock scanner. A developmentally engaging protocol was developed to help children stay still and acclimate to the scanner. In this "Statue Game," children were instructed to compete against a "statue" to see who could stay still longer. During the resting-state fMRI scan, participants were asked to stare at the picture of a "statue" and to "lay as still as a statue." Once the participant had completed the scanning procedure, the picture of the statue would move and the child would be informed that they had won the competition against the statue.

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up for a scheduled appointment, and 13 were not scheduled for visits because the target sample size was achieved.

2.3. rs-fMRI data acquisition

MRI data were acquired using a Siemens 3T TIM Trio MRI scanner (Siemens Healthcare, Erlangen, Germany) at Northwestern University's Center for Translational Imaging (CTI). The following parameters were: 40 axial slices, thickness/gap = 3/3 mm, TR/TE = 2500/20 ms, FA = 80°, in-plane resolution = 64 × 64, FOV = 896 mm × 840 mm, 244 volumes.

2.4. rs-fMRI data preprocessing

Data were processed using DPARSF and REST pipeline analysis (Song et al., 2011). First DICOM files were converted to NIFTI images. For all but two subjects, 244 volumes were kept and the remaining volumes were discarded. For two subjects, 20 of 244 volumes were excluded due to high motion. Then slice-timing and head motion correction, spatial normalization to the MNI (Montreal Neurological Institute) template and spatial resampling (3 mm × 3 mm × 3 mm) were performed. Data were smoothed (FWHM = 4 mm). Subjects with maximum head motion exceeding 3 mm in transition or 3° were excluded from further data analysis. Data were temporally band-pass filtered (0.01–0.08 Hz) to reduce physiological noise and the linear trend was removed.

2.5. Regional homogeneity (ReHo) analysis

To obtain ReHo values, on a voxel by voxel basis, Kendall's coefficient of concordance (KCC) of time series of a given voxel with those of its neighbor was calculated (Kendall and Gibbons, 1990). Voxels were considered to be neighbors if their corners were touching. The ReHo of each voxel was divided by the global mean value within the whole-brain mask. A larger ReHo value indicated higher regional synchronization. To explore correlations with the behavioral measures, 2nd level random-effects voxel-wise analyses were performed on ReHo maps (transformed KCC values) using SPM.

2.6. Resting-state functional connectivity (rs-FC) analysis

Following the ReHo analyses, resting-state functional connectivity (rs-FC) analyses were conducted. While ReHo measures similarity of the time courses within a cluster, rs-FC measures similarity of time courses between both proximal and distal brain regions. Regions identified as significantly related to stress based on ReHo were used as rs-FC seeds in whole-brain analysis. The time course from this ReHo seed region (using a 5 mm sphere around the significant peak) was extracted and spatially averaged, and voxel-wise whole brain correlation analyses were performed to generate rs-FC maps. The seed region itself was masked out of the connectivity maps. Correlations coefficients were converted to z-maps using Fisher's r-to-z transformation. To explore correlations with the behavioral measures, 2nd level random-effects voxel-wise analyses were performed on FC maps using SPM.

Statistical significance for the whole brain was defined using Monte Carlo simulations (using AFNI's AlphaSim program; http://afni.nimh.nih.gov/, with SPM's data smoothness parameters, FWHM = 14.3 13.9 11.2 mm). For the whole brain, to reach a corrected level threshold (alpha = 0.05), the clusters needed to contain 135 voxels with a voxel-wise threshold of $p = 0.005$.

3. Results

3.1. Behavioral performance

FSI perceived stress scores did not differ significantly across developmental periods (t (27) = .43, $p = .67$): Early Development $M = 2.4$ ($SD = 1.3$, *Range* = 1–5), and Concurrent $M = 2.3$ ($SD = 1.4$, *Range* = 1–5). Early and Concurrent Stress were significantly correlated with each other ($r = .52$, $p < .01$) but were not highly overlapping. Thus, when examining the effect of ELS during each development period, stress during the other developmental period was included as a covariate in the analyses.

Early vs. Concurrent Stress did not differ across the poverty or violence exposure groups nor were these scores correlated with child IQ ($M = 107$, $SD = 14$, *Range* = 71–127). However, parents of children with high vs. low Temper Loss reported higher Early, but not Concurrent Stress (see Table 1). Thus, children's Temper Loss status was included as a covariate in the following analyses. Because the time in between the assessment of ELS and resting-state fMRI varied by child, this factor was also included as a covariate. The statistical significance of the reported tests on rs-fMRI did not differ when child age at scan and child IQ were included as covariates considering an alpha of 0.05.

On the reversal-learning task, on average, before the switch children had 90% accuracy ($SD = 10\%$, *Range* = 58–100%), and 84% accuracy after the switch ($SD = 14\%$, *Range* = 33–97%). The average difference score was −.06 ($SD = .10$, *Range* = −.30 to .24). Reversal-learning was not related to Early or Concurrent Stress.

Table 1
Early and concurrent stress scores as a function of poverty, Temper Loss and violence exposure status.

	Poor ($n = 16$)	Non-poor ($n = 12$)	t
Early stress	2.6 (1.3)	2.2 (1.3)	0.93
Concurrent stress	2.1 (1.3)	2.7 (1.4)	1.14
	Low Temper Loss ($n = 14$)	High Temper Loss ($n = 14$)	
Early stress	1.9 (.9)	3 (1.4)	2.58*
Concurrent stress	2 (1.2)	2.6 (1.5)	1.24
	Violence exposed ($n = 10$)	Non-violence exposed ($n = 17$)	
Early stress	2.4 (1.3)	2.5 (1.3)	0.25
Concurrent stress	2.4 (1.7)	2.2 (1.3)	0.29

* $p < .05$.

3.2. Relation between resting-state homogeneity (ReHo) and ELS

We examined the relations between Early Stress and ReHo at the whole brain using 2nd level voxel-wise regression analyses, controlling for Concurrent Stress, Temper Loss group, and age difference between baseline and rs-fMRI scan. There were no significant clusters where ReHo was negatively correlated with Early Stress. Early Stress was positively related to ReHo in a cluster in the left middle frontal gyrus (MFG) (peak: $x = -42$, $y = 54$, $z = 15$, BA = 10, $z = 3.65$, $k = 136$ voxels) (Fig. 1). To ensure that the effects of perceived stress were over and above objective measures of stress, we ran analyses parallel to the one reported above controlling for the number of stressful acute events mothers reported. Early stress remained as a significant predictor controlling for number of stressful events and the previous covariates ($k = 216$, $p = 0.001$).

To confirm that relations to Early Stress were independent of other adversities (i.e., poverty and violence exposure), we extracted the average beta weight from the cluster in left MFG that showed a significant positive association with Early Stress, which served as our measure of early stress-related ReHo difference. The average ReHo in this cluster did not vary as a function of poverty group ($t(26) = 1.47$, $p = .15$) or as a function of violence exposure, ($t(25) = .64$, $p = .53$). A separate regression voxel-wise analysis across the whole brain including Early Stress exposure, Temper Loss and the interaction between Temper Loss and Early Stress did not reveal clusters significantly associated with the interaction term.

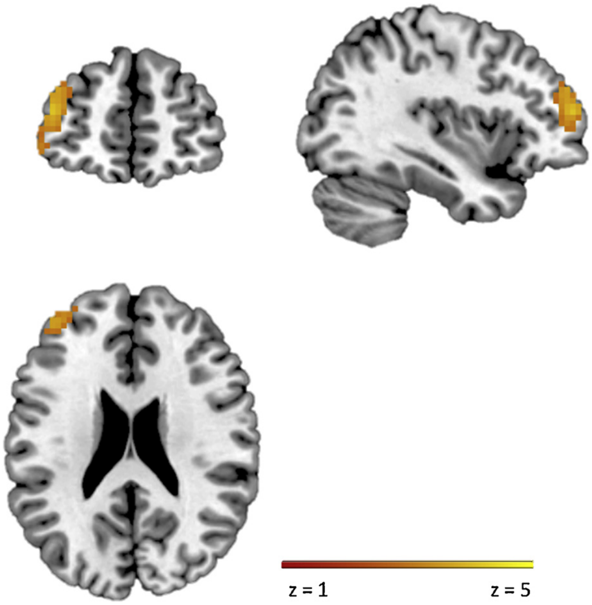

Fig. 1. Higher resting-state regional homogeneity in left middle frontal gyrus ($x=-42$, $y=54$, $z=15$) was associated with greater early-life stress. Statistical values are overlaid on coronal, sagittal, and axial slices of MNI-normalized anatomical brain.

We next examined the relation between Concurrent Stress and ReHo. There were no significant clusters where ReHo positively or negatively correlated with Concurrent Stress, controlling for Early Stress, Temper Loss group and age difference. Temper Loss group did not relate to ReHo positively or negatively at the whole brain level, controlling for Early or Concurrent Stress.

3.3. Relation between ReHo and reversal-learning

We also examined whether the ReHo differences in the left MFG were related to children's performance on the reversal-learning task. We extracted the average activation in the significant cluster. In a regression analysis, controlling for Concurrent Stress, Early Stress, Temper Loss group, and age difference, activation in the left MFG cluster was significantly negatively related to reversal-learning, i.e. pre-post switch difference score ($\beta=-.56$, $t(24)=-2.16$, $p=.04$, Fig. 2).[2] Thus, children with higher ReHo values in left MFG exhibited greater decrease in performance after the rule switch.

3.4. Resting-state functional connectivity (rs-FC)

For our rs-FC analyses, we used a 5 mm sphere around the peak of the cluster in left MFG identified above as related to Early Stress as our seed region. Whole brain connectivity analyses showed negative functional connectivity between our seed region and a cluster in right middle temporal gyrus/parahippocampal gyrus (peak: $x=42$, $y=18$, $z=-36$, BA = 38, $z=4.71$, $k=313$ voxels). Two clusters in left (peak: $x=-36$, $y=60$, $z=12$, BA = 10, $z=5.79$, $k=772$ voxels) and right middle frontal areas (peak: $x=48$, $y=51$, $z=9$, BA = 10, $z=4.91$, $k=171$ voxels) showed positive connectivity with the seed region (Fig. 3).

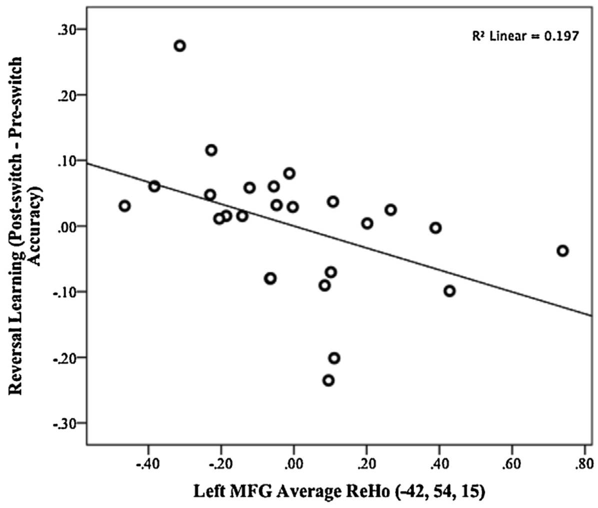

Fig. 2. Partial regression plot depicting the relation between resting-state regional homogeneity (ReHo) in left middle frontal gyrus (MFG) and reversal-learning (post-switch minus pre-switch accuracy), controlling for early stress, concurrent stress, Temper Loss, and age difference. Worse performance on reversal-learning was associated with higher regional homogeneity.

3.5. Relation between rs-FC and reversal-learning

We next examined if the relation between the seed regions and connected regions was related to reversal-learning. Controlling for age difference and Temper Loss, negative connectivity between the seed region and the right middle temporal/parahippocampal regions was positively related to the pre-post switch difference score ($\beta=.48$, $t(24)=2.39$, $p=.03$, Fig. 4).[3] Positive connectivity between the seed region and the bilateral middle frontal areas was not significantly related to reversal-learning ($\beta=-.09$, $p=.67$). Thus, increased negative coupling between the seed region and right middle temporal/parahippocampal areas was related to greater decrease in performance after the rule switch.

4. Discussion

To our knowledge, this is the first study to examine the relation of ELS exposure to resting-state fMRI in preschool age children. Our results showed that higher early, but not concurrent, ELS, is associated with greater ReHo of resting-state fMRI in left prefrontal cortex, i.e. left middle frontal gyrus, in preschool age children. Moreover, higher ReHo in this area and negative coupling between this area and right middle temporal/parahippocampal areas were negatively related to children's performance on a cognitive control task. Finally, the effects of ELS were independent of other aspects of stress experience including poverty, violence exposure and exposure to acute events.

We used ReHo as the primary measure of resting-state fMRI, which is considered to reflect intra-regional synchronization of spontaneous activity. Children with increased levels of early ELS exhibited increased ReHo in left middle frontal gyrus. Prefrontal regions, including middle frontal gyrus, are considered to be a part of task-positive network that is commonly activated during goal-oriented cognitive tasks (Kelly et al., 2008). Previous studies have reported increased resting ReHo and resting functional connectivity in frontal regions in adults and animals with

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[2] The relation remained significant using robust regression analysis, $\beta=-.28$, $p<0.01$.

[3] The relation remained significant using robust regression analysis, $\beta=.45$, $p=.02$.

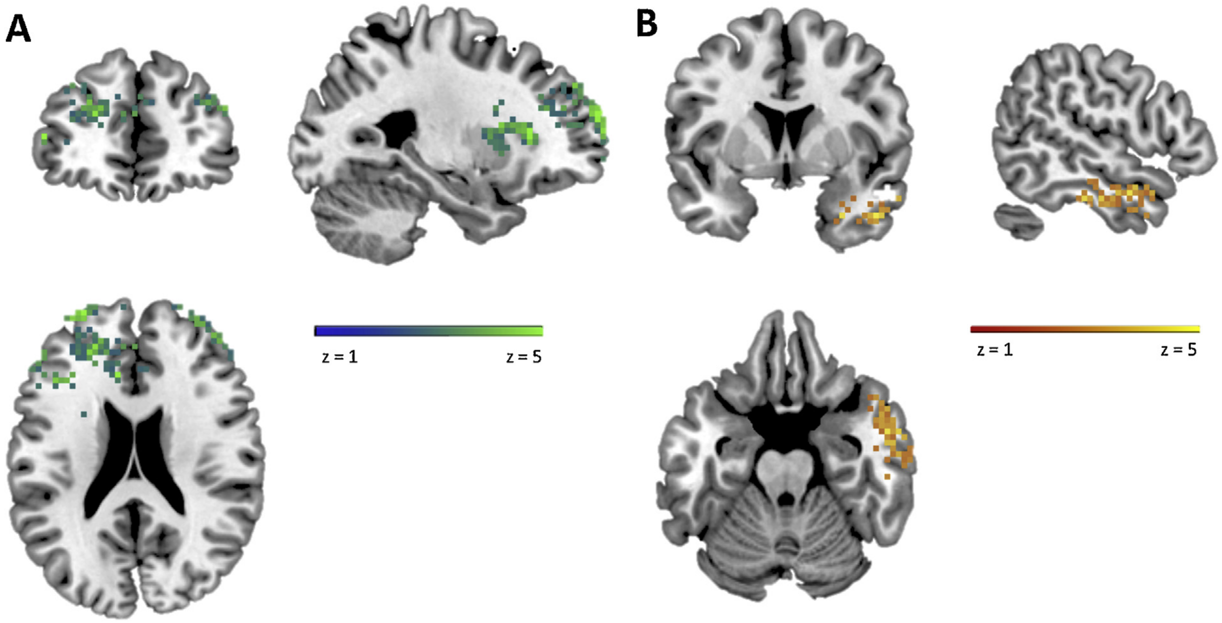

Fig. 3. Resting-state functional connectivity of the seed cluster with (A) left middle frontal gyrus to bilateral middle frontal areas (blue-green, $x = -36$, $y = 60$, $z = 12$ and $x = 48$, $y = 51$, $z = 9$) and (B) right middle temporal gyrus/parahippocampal areas (orange-red, $x = 42$, $y = 18$, $z = -36$). Statistical values are overlaid on coronal, sagittal, and axial slices of MNI-normalized anatomical brain.

concurrent or past extreme stress exposure (Bluhm et al., 2009; Ferris and Stolberg, 2010; Yin et al., 2012). We extend the existing literature by demonstrating the association of ELS exposure with prefrontal resting-state fMRI in young children, using robust measures of maternal perceived stress that account for developmental timing of exposure. This is particularly striking because it demonstrates that even exposure to normative levels of perceived stress well within the range of normal human experience is associated with disruptions in prefrontal resting-state activity and connectivity.

We also showed that higher ReHo in left middle frontal gyrus was associated with poorer cognitive control ability. Children who showed increased homogeneity in left middle frontal gyrus during rest took longer to switch from a rule they learned previously. Lesions to the prefrontal cortex selectively impair reversal-learning (Dias et al., 1996), and animal studies show deficits in reversal-learning is associated with ELS (Pryce et al., 2005). These prefrontal disruptions may presage the developmental pathway from ELS to later behavioral disinhibition and externalizing spectrum disorders (Clark et al., 2015; Lupien et al., 2009; Zucker et al., 2011). ELS might influence underlying neural systems that underlie behavioral disinhibition at a level not yet detectable by task-based measures, as ELS itself was not related to reversal-learning. The neural differences we observed during rest, in the absence of behavioral differences, add to a growing body of literature highlighting the importance of examining resting-state fMRI in children with atypical developmental pathways (Sonuga-Barke, 2014).

ReHo analyses were complemented with rs-FC analyses. rs-FC analyses revealed that the right middle frontal area, which was significantly related to stress, was positively connected to neighboring bilateral middle frontal areas and negatively connected to right middle temporal/parahippocampal areas. Middle temporal/parahippocampal areas are a central hub of the default-mode network, which shows coherent activity during rest and is suppressed during cognitive tasks (Raichle et al., 2001). Our results are consistent with a rich body of literature showing negative coupling between the default mode network and components of the task positive network, such as the prefrontal regions (Fox et al., 2005). Moreover, our findings revealed that stronger negative coupling was related to worse reversal-learning, suggesting that suppression of the default network during rest is associated with poorer cognitive control ability.

Our results suggest that exposure to chronic psychosocial stress might lead to increased neural reactivity to daily experiences, which in turn might be associated with the development of psychopathology (Blair and Raver, 2012; Ganzel et al., 2013). In the presence of increased levels of stress, children might show increased vigilance and reactivity to daily life events. These experiences and reactions might alter prefrontal regions, which subserve executive function and cognitive control. Our findings suggest that individuals with higher early stress exposure may engage the task positive network to a greater degree that results in suppression of the resting default network during rest. Although we show that these effects are related to reversal-learning measured outside the scanner, future studies should use functional tasks during scanning to examine relations between ELS and cognitive control and link these to clinical patterns.

Relation of ELS to increased ReHo in the middle frontal gyrus was independent of other stressors such as exposure to violence, acute life events or poverty. We measured ELS in terms of maternal perceptions of distress. ELS is a broad construct referring to multiple types of negative experiences in childhood. Our results suggest that different dimensions of ELS might differentially influence underlying neural systems (Sheridan and McLaughlin, 2014). Early psychosocial life stress might differentially influence prefrontal systems. Inequalities-related stressors might more strongly relate to differences in the neural basis of verbal processing involving inferior frontal and middle temporal gyri (Demir et al., 2015; Hackman and Farah, 2009). More extreme, adverse childhood experiences, such as maltreatment or abuse might differentially affect neural basis of emotional processing, such as amygdala (Sheridan and McLaughlin, 2014). Future studies should get a more nuanced understanding of different dimensions of ELS by using comprehensive models of stressors. Maternal stress perceptions may be particularly important for detecting patterns at the neural level, because these assessments tap into individual differences in

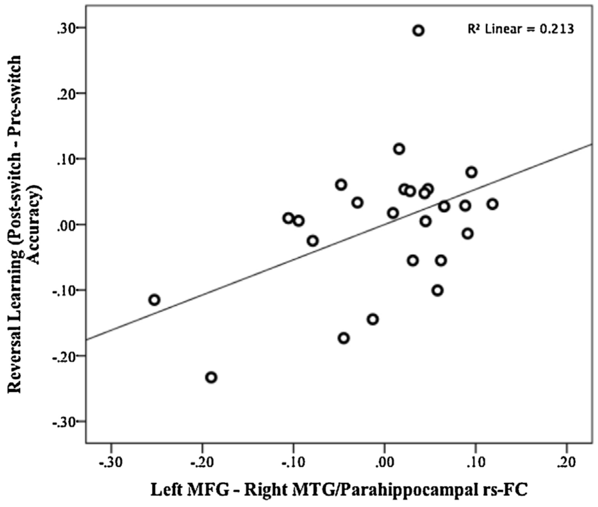

Fig. 4. Partial regression plot depicting the relation between resting-state functional connectivity (rs-FC) between left middle frontal gyrus (MFG) and right middle temporal(MTG)/parahippocampal area and reversal-learning (post-switch minus pre-switch accuracy), controlling for Temper Loss and age difference. Worse performance on reversal-learning was associated with greater negative coupling.

stress appraisal and embodiment (e.g. individuals may evaluate the same stressor as differentially stressful). Our results highlight the importance of differentiating different types of ELS when examining its relation to neurocognitive development. We showed these associations in a community sample of young children even when controlling for individual difference factors, such as IQ and Temper Loss.

Our results suggest that timing of ELS may also play a role in its associations to neural outcomes. The fact that early but not concurrent ELS related to rs-FMRI might be because the first years of life are times of rapid brain development; even mild psychosocial stressors experienced during early life could have pronounced effects on the developing brain (Lupien et al., 2009; Pechtel and Pizzagalli, 2011; Shonkoff et al., 2009). Brain regions that undergo protracted development, such as prefrontal areas, might be particularly vulnerable to ELS in the first years of life (Pechtel and Pizzagalli, 2011). Previous studies on humans and animals examining the effects of timing of ELS showed that effects of stress vary depending on the timing of the stressor and the measurement (De Bellis et al., 2002; de Kloet and Oitzl, 2003; Tottenham and Sheridan, 2009). The current study moves the field forward by showing that within the same group of young children earlier rather than later stress might play a more formative role in brain development, specifically of prefrontal areas. Unfortunately, we did not have measures of prenatal stress exposure. It is conceivable that the unique effects of early postnatal stress are a marker for prenatal stress (Sandman et al., 2012). Future studies, beginning in the prenatal period and utilizing multi-level measures of stress, will be important for establishing timing effects. Furthermore, our findings are based on associations between ELS and prefrontal functioning. Future studies using experimental designs or animal models should further explore if ELS is causally related to neural differences.

In sum, the current study to our knowledge for the first time examined the association of ELS during infancy to preschool age with resting-state fMRI in preschool to school-age children. Our results provided specificity regarding the neurocognitive systems associated with psychological ELS. Increased understanding of the effects of ELS on child brain-cognition relationships could inspire targeted preventions with relatively high impact owing to peak levels of neural and behavioral plasticity in early childhood during prodromal phases of clinical risk (Insel, 2009; Shonkoff et al., 2009).

Although our findings are not causal, they raise the possibility that modifying perceived stress, may be a mechanism for reducing risk during the early phase of the behavioral disinhibition pathway.

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References

Blair, C., Raver, C.C., 2012. Child development in the context of adversity: experiential canalization of brain and behavior. Am. Psychol. 67 (4), 309.

Bluhm, R.L., Williamson, P.C., Osuch, E.A., Frewen, P.A., Stevens, T.K., Boksman, K., Lanius, R.A., 2009. Alterations in default network connectivity in posttraumatic stress disorder related to early-life trauma. J. Psychiatry Neurosci. 34 (3), 187.

Burghy, C.A., Stodola, D.E., Ruttle, P.L., Molloy, E.K., Armstrong, J.M., Oler, J.A., Birn, R.M., 2012. Developmental pathways to amygdala-prefrontal function and internalizing symptoms in adolescence. Nat. Neurosci. 15 (12), 1736–1741.

Buss, C., Davis, E.P., Hobel, C.J., Sandman, C.A., 2011. Maternal pregnancy-specific anxiety is associated with child executive function at 6–9 years age. Stress 14 (6), 665–676.

Buss, C., Davis, E.P., Muftuler, L.T., Head, K., Sandman, C.A., 2010. High pregnancy anxiety during mid-gestation is associated with decreased gray matter density in 6–9-year-old children. Psychoneuroendocrinology 35 (1), 141–153.

Chugani, H.T., Behen, M.E., Muzik, O., Juhász, C., Nagy, F., Chugani, D.C., 2001. Local brain functional activity following early deprivation: a study of postinstitutionalized Romanian orphans. Neuroimage 14 (6), 1290–1301.

Clark, C., Espy, K., Wakschlag, L., 2015. How do adverse early life exposures work together? Joint consideration of pre-postnatal to psychosocial stress and tobacco in behavioral disinhibition pathways. Submitted manuscript.

Crowley, M.J., Mayes, L.C., 2004. The Candy Reversal Learning Task. Social Affective Neuroscience Laboratory (SANDL). Yale Child Study Center.

De Bellis, M.D., Keshavan, M.S., Shifflett, H., Iyengar, S., Beers, S.R., Hall, J., Moritz, G., 2002. Brain structures in pediatric maltreatment-related posttraumatic stress disorder: a sociodemographically matched study. Biol. Psychiatry 52 (11), 1066–1078.

Demir, Ö.E., Prado, J., Booth, J.R., 2015. Parental socioeconomic status and the neural basis of arithmetic: differential relations to verbal and visuo-spatial representations. Dev. Sci.

Dias, R., Robbins, T.W., Roberts, A.C., 1996. Dissociation in prefrontal cortex of affective and attentional shifts. Nature 380 (6569), 69–72.

Dodge, K.A., Pettit, G.S., Bates, J.E., 1994. Socialization mediators of the relation between socioeconomic status and child conduct problems. Child Dev. 65, 649–665.

Eluvathingal, T.J., Chugani, H.T., Behen, M.E., Juhász, C., Muzik, O., Maqbool, M., Makki, M., 2006. Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. Pediatrics 117 (6), 2093–2100.

Elliott, C.D., 1983. Differential Abilities Scale. Psychological Corp., San Antonio.

Finger, E.C., Marsh, A.A., Mitchell, D.G., Reid, M.E., Sims, C., Budhani, S., Blair, J.R., 2008. Abnormal ventromedial prefrontal cortex function in children with psychopathic traits during reversal learning. Arch. Gen. Psychiatry 65 (5), 586–594.

Ferris, C.F., Stolberg, T., 2010. Imaging the immediate non-genomic effects of stress hormone on brain activity. Psychoneuroendocrinology 35 (1), 5–14.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc. Natl. Acad. Sci. U. S. A. 102 (27), 9673–9678.

Ganzel, B.L., Kim, P., Gilmore, H., Tottenham, N., Temple, E., 2013. Stress and the healthy adolescent brain: evidence for the neural embedding of life events. Dev. Psychopathol. 25 (4 pt 1), 879–889.

Glover, V., 2011. Annual research review: prenatal stress and the origins of psychopathology: an evolutionary perspective. J. Child Psychol. Psychiatry 52 (4), *356*–367.

Graignic-Phillipe, R., Dayan, J., Chokron, S., Jacquet, A.-Y., Tordman, S., 2014. Effects of prenatal stress on fetal and child development: a critical literature review. Neurosci. Biobehav. Rev. 43, 137–162.

Hackman, D.A., Farah, M.J., 2009. Socioeconomic status and the developing brain. Trends Cogn. Sci. 13 (2), 65–73.

Hanson, J.L., Chung, M.K., Avants, B.B., Shirtcliff, E.A., Gee, J.C., Davidson, R.J., Pollak, S.D., 2010. Early stress is associated with alterations in the orbitofrontal cortex: a tensor-based morphometry investigation of brain structure and behavioral risk. J. Neurosci. 30 (22), 7466–7472.

Hanson, J.L., Chung, M.K., Avants, B.B., Rudolph, K.D., Shirtcliff, E.A., Gee, J.C., Pollak, S.D., 2012. Structural variations in prefrontal cortex mediate the relationship between early childhood stress and spatial working memory. J. Neurosci. 32 (23), 7917–7925.

Insel, T.R., 2009. Translating scientific opportunity into public health impact: a strategic plan for research on mental illness. Arch. Gen. Psychiatry 66 (2), 128–133.

Jaffee, S.R., Caspi, A., Moffitt, T.E., Polo-Tomas, M., Price, T.S., Taylor, A., 2004. The limits of child effects: evidence for genetically mediated child effects on corporal punishment but not on physical maltreatment. Dev. Psychol. 40 (6), 1047.

Kelly, A.C., Uddin, L.Q., Biswal, B.B., Castellanos, F.X., Milham, M.P., 2008. Competition between functional brain networks mediates behavioral variability. Neuroimage 39 (1), 527–537.

Kendall, M., Gibbons, J.D., 1990. Correlation Methods. Oxford University Press, Oxford, pp. 1990.

Kim, C., Cilles, S.E., Johnson, N.F., Gold, B.T., 2012. Domain general and domain preferential brain regions associated with different types of task switching: a meta-analysis. Hum. Brain Mapp. 33 (1), 130–142.

Kim, P., Evans, G.W., Angstadt, M., Ho, S.S., Sripada, C.S., Swain, J.E., Phan, K.L., 2013. Effects of childhood poverty and chronic stress on emotion regulatory brain function in adulthood. Proc. Natl. Acad. Sci. U. S. A. 110 (46), 18442–18447.

Long, X.Y., Zuo, X.N., Kiviniemi, V., Yang, Y., Zou, Q.H., Zhu, C.Z., Jiang, T.Z., Yang, H., Gong, Q.Y., Wang, L., Li, K.C., Xie, S., Zang, Y.F., 2008. Default mode network as revealed with multiple methods for resting-state functional MRI analysis. J. Neurosci. Methods. 171 (2), 349–355.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10 (6), 434–445.

Mackey, A.P., Raizada, R.D.S., Bunge, S.A., 2012. Environmental influences in prefrontal development. In: Stuss, D.T., Knight, R.T. (Eds.), Principles of Frontal Loba Function. , pp. 146–163.

Mehta, M.A., Golembo, N.I., Nosarti, C., Colvert, E., Mota, A., Williams, S.C., Sonuga-Barke, E.J., 2009. Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the English and Romanian Adoptees study pilot. J. Child Psychol. Psychiatry 50 (8), 943–951.

Mueller, S.C., Maheu, F.S., Dozier, M., Peloso, E., Mandell, D., Leibenluft, E., Ernst, M., 2010. Early-life stress is associated with impairment in cognitive control in adolescence: an fMRI study. Neuropsychologia 48 (10), 3037–3044.

Pechtel, P., Pizzagalli, D.A., 2011. Effects of early life stress on cognitive and affective function: an integrated review of human literature. Psychopharmacology (Berl.) 214 (1), 55–70.

Pryce, C.R., Rüedi-Bettschen, D., Dettling, A.C., Weston, A., Russig, H., Ferger, B., Feldon, J., 2005. Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. Neurosci. Biobehav. Rev. 29 (4), 649–674.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc. Natl. Acad. Sci. U. S. A. 98 (2), 676–682.

Sandman, C.A., Davis, E.P., Glynn, L.M., 2012. Prescient human fetuses thrive. Psychol. Sci. 23 (1), 93–100.

Seeley, W.W., Menon, V., Schatzberg, A.F., Keller, J., Glover, G.H., Kenna, H., Greicius, M.D., 2007. Dissociable intrinsic connectivity networks for salience processing and executive control. J. Neurosci. 27 (9), 2349–2356.

Sheridan, M.A., McLaughlin, K.A., 2014. Dimensions of early experience and neural development: deprivation and threat. Trends Cogn. Sci. 18 (11), 580–585.

Shonkoff, J.P., Boyce, W.T., McEwen, B.S., 2009. Neuroscience, molecular biology, and the childhood roots of health disparities: building a new framework for health promotion and disease prevention. J. Am. Med. Assoc. 301 (21), 2252–2259.

Sonuga-Barke, 2014. Far from idle: four ways in which growing knowledge of the 'resting' brain is transforming our understanding of the causes of childhood disorder. J. Child Psychol. Psychiatry 55 (12), 1297–1299.

Sripada, R.K., King, A.P., Garfinkel, S.N., Wang, X., Sripada, C.S., Welsh, R.C., Liberzon, I., 2012. Altered resting-state amygdala functional connectivity in men with posttraumatic stress disorder. J. Psychiatry Neurosci. 37 (4), 241.

Song, X.W., Dong, Z.Y., Long, X.Y., Li, S.F., Zuo, X.N., Zhu, C.Z., Zang, Y.F., 2011. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS ONE 6 (9), e25031.

Straus, M.A., Hamby, S.L., Boney-McCoy, S., Sugarman, D.B., 1996. The revised conflict tactics scales (CTS2) development and preliminary psychometric data. J. Fam. Issues 17 (3), 283–316.

Teicher, M.H., Andersen, S.L., Polcari, A., Anderson, C.M., Navalta, C.P., Kim, D.M., 2003. The neurobiological consequences of early stress and childhood maltreatment. Neurosci. Biobehav. Rev. 27 (1), 33–44.

Tottenham, N., Sheridan, M.A., 2009. A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. Front. Hum. Neurosci., 3.

Wakschlag, L.S., Choi, S.W., Carter, A.S., Hullsiek, H., Burns, J., McCarthy, K., Briggs-Gowan, M.J., 2012. Defining the developmental parameters of temper loss in early childhood: implications for developmental psychopathology. J. Child Psychol. Psychiatry 53 (11), 1099–1108.

Wakschlag, L.S., Briggs-Gowan, M.J., Choi, S.W., Nichols, S.R., Kestler, J., Burns, J.L., Henry, D., 2014. Advancing a multidimensional, developmental spectrum approach to preschool disruptive behavior. J. Am. Acad. Child Adolesc. Psychiatry 53 (1), 82–96, e83.

Wakschlag, L., Estabrook, R., Henry, D., Burns, J., Perlman, S., Pine, D., Leibenluft, E., Briggs-Gowan, M., 2015. Clinical application of a dimensional approach: The normal:abnormal spectrum of irritability in preschoolers. J. Am. Acad. Child Adolesc. Psychiatry 54, 626–634.

Woon, F.L., Hedges, D.W., 2008. Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis. Hippocampus 18 (8), 729–736.

Yin, Y., Jin, C., Eyler, L.T., Jin, H., Hu, X., Duan, L., Zheng, H., Feng, B., Huang, X., Shan, B., Gong, Q., Li, L., 2012. Altered regional homogeneity in post-traumatic stress disorder: a restingstate functional magnetic resonance imaging study. Neurosci. Bull. 28 (5), 541–549.

Zucker, R.A., Heitzeg, M.M., Nigg, J.T., 2011. Parsing the undercontrol–disinhibition pathway to substance use disorders: a multilevel developmental problem. Child Adolesc. Perspect. 5 (4), 248–255.