Biological Psychology 145 (2019) 198–210

Contents lists available at ScienceDirect

Biological Psychology

journal homepage: www.elsevier.com/locate/biopsycho

Chronic harsh parenting and anxiety associations with fear circuitry function in healthy adolescents: A preliminary study

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ARTICLE INFO

Keywords:
Fear conditioning
Connectivity
Amygdala
Adolescent
Parenting

ABSTRACT

Previous studies have reported altered fear circuitry function during fear conditioning in highly anxious individuals and in adults with a history of severe childhood adversity; less is known regarding younger populations and more common forms of adversity. We investigated fear circuitry functioning in healthy youths with histories of high (HH) or low (LH) chronic harsh parenting and high (HA) or low (LA) anxiety levels. 84 youths aged 13–16 performed an fMRI fear conditioning task. HH displayed decreased selective medial temporal lobe deactivations to CS+ > CS− relative to LH. In addition, we found less amygdala-insula connectivity in HH vs LH. Interestingly, we observed distinct patterns of anxiety differences in amygdala-rostral ACC connectivity and subjective fear ratings depending on harsh parenting levels, suggesting a history of harsh parenting is linked with unique neural and behavioral anxious manifestations, which are different from anxiety manifestations in a context of low adversity.

1. Introduction

Extensive evidence links early adversity with impaired socio-emotional development and long-lasting psychopathology (Hart & Rubia, 2012; MacMillan et al., 1999; McCrory & Viding, 2015; McCrory, Gerin, & Viding, 2017). Most studies to date on adverse caregiving have focused on maltreated or neglected children. However, youths exposed to harsh parenting, a "milder" form of maltreatment defined by hostile child-rearing behaviors (e.g. mild physical punishment, being verbally abusive) that is still considered acceptable and is highly prevalent in several countries including Canada and the United States (Clément, Bernèche, Fontaine, & Chamberland, 2012; Gershoff, 2008), are also at a high risk of anxiety disorders and other negative socio-emotional outcomes (McCrory & Viding, 2015; McLeod, Wood, & Weisz, 2007).

In both adults and youths, early-life adversity is related to greater sensitivity to threatening cues (Hart & Rubia, 2012; McCrory et al., 2017). Such altered threat processing is also closely associated with anxiety symptoms (Hofmann, Ellard, & Siegle, 2012). These links may be explained by alterations in the "fear circuitry", a neural system particularly sensitive to early-life adversity (Hart & Rubia, 2012). This system plays a key role in the learning and expression of fear, which rely mainly on activity in medial temporal lobe structures (i.e. amygdala and anterior hippocampus), the insula, and the dorsal anterior cingulate cortex (dACC) and in fear regulation, which is associated with ventromedial prefrontal cortex (vmPFC) activity (LeDoux, 2000; Marek, Strobel, Bredy, & Sah, 2013; Milad & Quirk, 2012; Simmons et al., 2013; for a more comprehensive description of the fear circuitry, see Marek et al., 2013; Tovote, Fadok, & Luthi, 2015). In this study, we investigated fear circuitry function in youths with a history of high and low maternal harsh parenting displaying high or low levels of chronic,

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https://doi.org/10.1016/j.biopsycho.2019.03.019
Received 14 February 2018; Received in revised form 25 March 2019; Accepted 27 March 2019
Available online 29 March 2019

non-clinical anxiety, so as to shed light on the neural mechanisms that link and/or distinguish early adversity and trait anxiety in healthy adolescents.

Fear conditioning tasks are extensively used in the study of fear circuitry function in humans and animals as their underlying mechanisms are closely involved in the pathophysiology and treatment of anxiety disorders (Lissek et al., 2005; Lissek, 2012; McGuire et al., 2016; Milad & Quirk, 2012). Fear conditioning is a process by which a conditioned stimulus (CS+; e.g., a neutral face), after repeated pairing with an aversive unconditioned stimulus (US; e.g., electrical shocks), elicits a conditioned fear response. In discrimination fear conditioning, a second CS, the CS-, is never paired with the US, and serves as a safety signal; conditioned responses are measured as the difference between CS + and CS-.

Conditioning paradigms have clearly demonstrated alterations of fear processing in individuals with anxiety disorders (Lissek et al., 2005), as well as in individuals at-risk for anxiety disorders, including those with early adversity histories and those with elevated trait anxiety. Trait anxiety is a predisposition to experience fear and anxiety across a wide range of situations (Gidron, 2013) that is commonly encountered in individuals with anxiety disorders (Kampman, Viikki, & Leinonen, 2017). Hence, different features of trait anxiety may contribute to the development of anxiety disorders and may affect the course and severity of the disorder (for a detailed review, see Kampman et al., 2017). Whereas an overgeneralization of fear responses to safety signals (CS-) and increased responses to both CSs are typically reported in clinically anxious individuals (Lissek, 2012; Lissek et al., 2005), increased subjective distress levels, physiological responses (e.g. skin conductance rates [SCRs], startle reflex), and medial temporal lobe responses to CS+ (or to CS + vs. CS-) have been reported in healthy individuals with high trait anxiety (Craske et al., 2008; Indovina, Robbins, Nunez-Elizalde, Dunn, & Bishop, 2011; Jovanovic et al., 2014). Similar patterns have been reported in adults with post-traumatic stress disorder (PTSD) related to a history of early adversity (e.g., Bremner et al., 2005). Although very few studies have investigated fear conditioning mechanisms in youths with early adversity histories, a recent study demonstrated stronger hippocampus activation to CS + relative to CS- in previously institutionalized youths relative to controls, as well as greater subjective threat-safety discrimination in those with higher trait anxiety levels (Silvers et al., 2016). Moreover, studies employing other emotion-evoking stimuli (e.g. facial pictures) have shown increased responses to threat in the amygdala, hippocampus and insula in youths with histories of early adversity relative to controls, although most of these children displayed normal levels of anxiety (for reviews, see Hein & Monk, 2017; McCrory et al., 2017).

In addition, reduced amygdala-PFC coupling has been reported in adults with a history of childhood trauma (e.g., Herringa et al., 2013), an anxiety disorder (A. Etkin, Prater, Hoeft, Menon, & Schatzberg, 2010), or both (e.g., Birn, Patriat, Phillips, Germain, & Herringa, 2014), as well as in maltreated children and adolescents (Thomason et al., 2015). Conversely, increased amygdala-vmPFC connectivity has been observed in psychiatrically healthy adults and adolescents at high risk of anxiety disorders (e.g., Hardee et al., 2013; Tzschoppe et al., 2014), and has also been associated with reduced anxiety symptoms (e.g., Birn et al., 2014; Herringa et al., 2013; Thomason et al., 2015). Insula findings are less clear, with some studies reporting increased amygdala-insula connectivity in populations with anxiety and/or a history of early adversity (e.g., Thomason et al., 2015), while others report opposite patterns (e.g., A. Etkin et al., 2010; Van der Werff et al., 2013).

Taken together, these findings suggest a history of early and chronic adversity, including harsh parenting, as well as high trait anxiety levels, may be associated with altered fear processing both at the behavioral and neural levels. To date, however, very few studies have examined fear conditioning mechanisms in youths with histories of early adversity. Most of the previous studies employed other emotional paradigms and used small or heterogeneous samples that mixed individuals with early-life adversity of different origins and with comorbid psychiatric symptoms. Importantly, most previous studies investigated the correlates of early adversity without controlling for trait anxiety levels and without examining joint associations or interactions between adversity and trait anxiety. Thus, to date, it is difficult to disentangle the correlates of early adversity from influences of elevated trait anxiety levels. Better identifying neural substrates specific to harsh parenting or trait anxiety and potential interactions may inform future preventative interventions in high-risk youths. For instance, if anxiety symptoms in a context of harsh parenting are linked with neural substrates different from those observed in a context of low adversity, this may suggest the need for alternative intervention forms. Finally, very few studies examined "milder" but also highly prevalent forms of adversity such as harsh parenting. Hence, whether harsh parenting practices are related to fear circuitry alterations remains unclear for the moment.

In the present study, we examined the specific and joint associations of early and chronic life adversity – in the unique form of chronic high harsh parenting – and chronic, non-clinical, trait anxiety levels, both measured yearly from 2.5 to nine years old, with fear circuitry function and connectivity in a sample of 84 healthy adolescents. Participants were split into four groups according to maternal harsh parenting levels and anxiety levels: high harsh parenting and high anxiety levels (HH/HA), high harsh parenting and low anxiety levels (HH/LA), low harsh parenting and high anxiety levels (LH/HA), and low harsh parenting and low anxiety levels (LH/LA). Youths underwent a fear conditioning task using a pediatrically safe US, the Screaming Lady task (Lau et al., 2008), shown to be successful in triggering fear responses in youths (Chauret et al., 2014; Lau et al., 2008).

We expected greater discrimination conditioning, reflected by increased fear ratings, SCRs responses, and amygdala, anterior hippocampus, and insula activations to CS+ > CS-, in youths with high harsh parenting and/or anxiety levels (HH/HA, HH/LA, and LH/HA) relative to LH/LA. We had no a priori hypotheses regarding *specific* associations with harsh parenting or anxiety, given the exploratory nature of the study. In terms of connectivity, decreased amygdala-vmPFC connectivity was expected in high relative (HH/HA and LH/HA) to low anxiety groups (HH/LA and LH/LA). In addition, we expected to observe interactions between harsh parenting and trait anxiety such that youths in the HH/LA group would show increased amygdala-vmPFC connectivity relative to youths in the LH/LA group.

2. Materials and methods

2.1. Participants

Participants were recruited in two related cohorts from two prospective longitudinal studies: *In 2001, I was 5 years old* (Jetté, Desrosiers, & Tremblay, 1998) and *The Quebec Longitudinal Study of Children's Development* (Jetté & Des Groseilliers, 2000). Recruitment was performed through the Research Unit on Children's Psychosocial Maladjustment in collaboration with the Quebec Statistical Institute ("Institut de la Statistique du Québec"). These cohorts include a total of 2746 mother-child dyads; children were representative of singletons born in the province of Quebec (Canada) between 1996 and 1998 (children from the far North, Cree or Inuit regions, and from aboriginal reservations were excluded). Longitudinal data regarding youths and their parents' socio-demographic profile, psychological development (including anxiety levels), familial interactions (including harsh parenting practices) and health status were collected yearly from the time the youths were 5 months old.

2.1.1. Assignment to groups

For the purpose of this study, we narrowed down the cohorts to 1761 possible participants, after selecting only those for whom data on their anxiety profile and their parents' harsh parenting practices were collected at least three times between the ages of 2.5 and 9 years (the

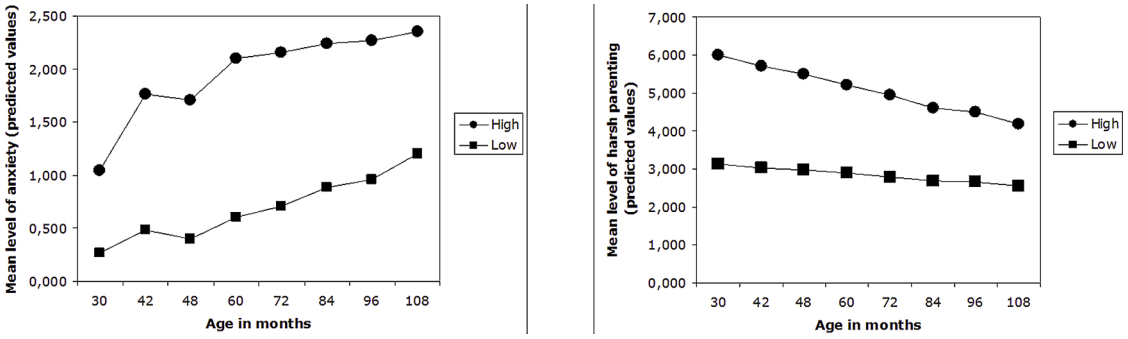

Fig. 1. Trajectory groups for anxiety (high (50%) and low (50%)) and harsh parenting (high (52%) and low (48%)) (n = 1761).

most recent data point at which both variables were measured). At least one of those measurements had to have been taken in the last two times they were evaluated, at ages 8 and 9 years. A developmental trajectory methodology (Nagin, 2005) was employed to determine how to distribute youths according to maternal harsh parenting and child anxiety levels into the four following groups: LH/LA, LH/HA, HH/LA and HH/HA. This is an empirical method that identifies groups of children who follow similar developmental patterns over time. When employing this method, a semi-parametric, group-based mixture model is computed using all available data points across time. The model assigns individuals to trajectory groups on the basis of a posterior probability rule (Nagin, 2005). Developmental trajectories were computed based on youths' anxiety symptoms and maternal harsh parenting practices collected across time (i.e., when youths were 2.5–9 years old). A two-group (high vs. low) solution was selected as the best-fitting model that minimized the Bayesian Information Criterion (Nagin, 2005) for both youths' anxiety symptoms and mothers' harsh parenting practices (see Fig. 1). Developmental trajectories for both variables were then computed simultaneously to get a valid estimation of the proportion of youths in each of the four groups of interest. The model reported that, of the 1761 youths in QLSCD cohorts, 30% were in the /LA/LH group (n = 548), 20% in the HH/LA group (n = 347), 18% in the LH/HA group (n = 306) and 32% in the HH/HA group (n = 560). See Fig. 1 for trajectory groups.

2.1.2. Inclusion and exclusion criteria measures

Since screening 1761 youths was impossible for financial and practical reasons, only a proportion of youths in all four groups were screened. Hence, for the LH/LA and HH/HA groups, the screening was planned for the 30% of youths with the highest probability of belonging to these trajectory-defined groups (n = 165 and 168, respectively). Given that the HH/LA and LH/HA groups were smaller than the other groups, we planned on screening the top 45% and 60% respectively (n = 156 and 183, respectively). These numbers were selected in an attempt to reach a target number of 30 participants per group. Youths who met the inclusion criteria and had the highest probability of belonging to any of the four cells were included in the study first, until completion of the study sample. To ensure that their current profile matched the group they supposedly belonged to, selected youths were re-evaluated on both variables using the SCARED anxiety level questionnaire (Birmaher et al., 1997) and the parenting practices questionnaire (NLSCY; Statistics Canada, 1995; Boivin et al., 2005). Exclusion criteria for youths in this study included: (a) current or past neurological or psychiatric diagnostic, as measured by the *Kiddie Schedule for Affective Disorders and Schizophrenia* (K-SADS) (Kaufman et al., 1997), a structured clinical psychiatric assessment based on DSM-IV criteria that was administered to all youths and their parents separately; (b) current use of psychotropic medication, or current psychological treatment, for psychiatric illness; (c) past head trauma; (d) past or current abuse; (e) contraindications for MRI (e.g. braces); and (f) Verbal IQ scores < 70 assessed with the *Peabody Picture Vocabulary Test-Revised* (PPVT) in English (Dunn & Dunn, 1981) or French (Dunn, Theriault-Whalen, & Dunn, 1993)).

2.1.3. Final sample

A total of 112 youths and their parents agreed to participate in the study. Of these, 10 were excluded because of MRI contraindications, and 8 were excluded after screening because of the presence of a

Table 1
Descriptive and psychological characteristics of the final sample per group.

	LH		HH		Main effect of HP	Main effect of A	HP*A / χ^2 group comparison
	LA	HA	LA	HA	p =	p =	p =
N	21	19	23	21			
Sex (female/male)	10/11	14/5	11/12	10/11	.260	.260	.262
Age	14.10 (0.75)	13.92 (0.63)	13.87 (0.54)	14.21 (0.74)	.853	.608	.082
Tanner stage	3.76 (0.71)	4.07 (0.75)	3.88 (0.83)	4.05 (0.63)	.113	.605	.213
Verbal IQ (PPVT-R)	111 (14.49)	111 (13.88)	108 (9.97)	103 (13.80)	.057	.282	.359
SES	34.93 (9.10)	36.84 (14.94)	37.07 (10.38)	43.29 (12.92)	.104	.123	.412
CDI T-scores	44.14 (5.92)	44.26 (6.31)	44.56 (5.89)	46.58 (6.70)	.208	.269	.342
Current harsh parenting scores	6.86 (2.29)	6.05 (1.72)	8.35 (1.99)	8.43 (2.36)	< .001	.435	.340
Current anxiety levels (SCARED-R parent version)	8.07 (8.61)	12.45 (7.03)	11.24 (7.21)	16.09 (10.67)	.163	.014	.892
Current anxiety levels (SCARED-R child version)	16.86 (7.66)	20.39 (8.77)	19.20 (10.46)	19.48 (10.75)	.589	.394	.458
Valid SCR data	17	11	18	17	.313	.313	.274
Mean STS displacement	0.11 (0.05)	0.11 (0.07)	0.11 (0.06)	0.29 (0.78)	.301	.305	.311

Note. Means and (standard deviations). Significant p values are indicated in bold characters, LH = Low harsh parenting; HH = High harsh parenting; LA = Low anxiety; HA = High anxiety; HP = Harsh parenting; A = Anxiety; PPVT-R = Peabody Picture Vocabulary Test-Revised; SES = Socio-Economic Status; CDI = Child Depression Inventory; STS = scan-to-scan.

psychiatric disorder. In total, 94 youths aged between 13 and 16 years at the time of testing went through the MRI procedure. Of these youth, 91 had complete functional MRI data. The data of a further seven youths were removed because of excessive motion during the scanning sessions (> 3.5 mm). Hence, the final sample was comprised of 84 youths (see Table 1 for the sample's descriptive and psychological characteristics).

2.2. Measures

2.2.1. Harsh parenting practices

A questionnaire including different subscales investigating parenting practices (e.g., maternal self-efficacy, perceived parental impact, parental overprotection) was administered to mothers when youths were 30, 42, 48, 60, 72, 96 and 108 months old and at the time of testing. Questions on harsh parenting were selected from the Hostile/Ineffective scale used in the National Longitudinal Survey of Children and Youth (NLSCY; Statistics Canada, 1995) and from the Parental Cognitions and Conduct Toward the Infant Scale (PACOTIS; Boivin et al., 2005). Regarding harsh parenting practices, mothers were asked to rate themselves on a frequency scale indicating if they never (0), less than half the time (1), half the time (2), more than half the time (3) or all the time (4): "got angry when punishing your child"; "spanked your child when he/she was difficult"; "raised your voice, scolded or yelled at your child when he/she broke the rules or did things he/she was not supposed to do"; and "used physical punishment (e.g., shaking) when he/she broke the rules or did things he/she was not supposed to do". These chosen items have been validated by a panel of 15 expert clinical and developmental psychologists for content, have been used in several large population studies (e.g., Battaglia et al., 2016; Boivin et al., 2005; Galéra et al., 2014; Pierce et al., 2010), and have shown adequate psychometric properties for the evaluation of maternal harsh parenting practices towards infants up to school-age children (Boivin et al., 2005; Boyle et al., 2004; Pierce et al., 2010). Scores on each of the four items were added so that final scores ranged between 0 and 16. The internal consistency value (alphas) was .58, .44, .81, .73, .78, .73, .62, at 30, 42, 48, 60, 72, 96 and 108 months, respectively and .98 at moment of testing.

2.2.2. Anxiety levels

Three items evaluating youths' anxiety symptoms were selected from the anxious/depressed and emotionally reactive subscales of the *Child Behavior Checklist* (Achenbach, 1991). Mothers indicated the frequency of anxious manifestations on a two-point scale ranging from 0 (never) to 2 (often). These items have been used in large population studies and have shown adequate psychometric properties for the evaluation of anxiety levels in early childhood up to adolescence (Boyle et al., 2004; Cote et al., 2009; Galéra et al., 2014). Mothers answered questions when youths were 30, 42, 48, 60, 72, 84, 96 and 108 months old. Coefficients of internal consistency were.78,.67,.78,.72,.80,.74,.75, and .81 at 30, 42, 48, 60, 72, 84, 96 and 108 months, respectively, in the present sample. Current anxiety levels were re-rated by the mothers and youths at the time of scanning with the *Screen for Child Anxiety Related Emotional Disorders* (SCARED), a questionnaire that measures five dimensions of anxiety symptoms in children and adolescents and that has been proven to possess adequate psychometric properties (Birmaher et al., 1997). Internal consistency value was .79 for both the child and parent versions.

2.2.3. Other descriptive characteristics

Socio-economic status (SES) was measured using the four-factor Hollingshead scale, which combines scales for maternal and paternal education and occupation (Hollingshead, 1973). Pubertal stage was assessed with the *Tanner scale* (Tanner, 1962). Finally, to rule out the presence of depression, depression symptoms were assessed with the *Child Depression Inventory* (CDI) (Kovacs, 1984).

2.3. Procedure

2.3.1. Recruitment and screening

All procedures were approved by the Research Ethics Boards of the Research Center of the Ste-Justine University Hospital, Montreal, Canada, and of the *Institut Universitaire de Gériatrie de Montréal* (IUGM), Canada. First, letters were sent to the families to explain the aims and conditions of the study. Participants' mothers were then contacted by telephone, and home interviews were conducted with interested families to screen for possible exclusion criteria and complete the KSADS interview and all questionnaires. Participants that fulfilled both the inclusion and exclusion criteria were invited to the fMRI session.

2.3.2. Experimental design

We used a 17-minute paradigm comprised of two phases: fear conditioning and extinction (Lau et al., 2008, 2011). Only fear conditioning will be presented in this article. Participants saw headshots of two actresses presenting neutral emotional expressions. For each participant, one actress was randomly selected to serve as the conditioned stimulus (CS+), and the other served as the safety signal (CS-). The CS + was paired on 50% of the trials with the unconditioned stimulus (US), which consisted of a photograph of the actress selected for the CS + depicting a fearful expression and presented simultaneously with a 90 dB shrieking scream. A partial reinforcement contingency ratio was used to prevent habituation to the US (Mackintosh, 1974). The other actress served as a conditioned stimulus unpaired with the aversive US (CS-). Participants were unaware of the CS+ – US association prior to the experiment. Subjective fear ratings and skin conductance responses (SCRs) were recorded during the whole procedure, at each presentation of the stimuli.

A total of 56 stimuli were presented during the conditioning phase. Events were comprised of one of the three following events: CS +-paired with the US (n = 14), CS+-unpaired (n = 14), and CS- (n = 14). During the CS + paired events, a neutral face was presented (3-s), followed by a rating response (3-s), and the US (1.1-s). During the CS+-unpaired and CS- events, a neutral face was presented (3-s), followed by a rating response (3-s). Thus, both CS+-unpaired and CS- events were presented for a duration of 6 s, while the CS+-paired with the US were presented for 7.1 s; inter-stimulus intervals lasted 3,4, 5, 6, 8, 10, or 12 s. See Fig. 2 for a schematic depiction of the experimental design.

2.3.3. Scanning procedure

Functional MRI scans were performed at the Montreal Geriatric University Institute. Before testing, participants had been familiarized with the MRI environment and with the experimental paradigm in a

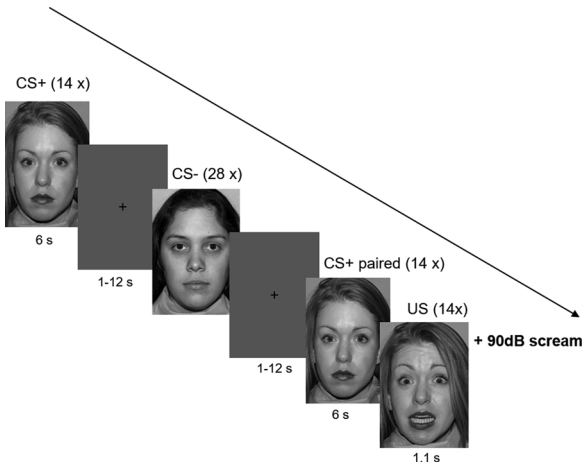

Fig. 2. Schematic depiction of the fear conditioning task; CS+: conditioned stimulus, CS- safety cue, US: unconditioned stimulus.

mock scanner, to ensure that they understood how to rate the pictures. To prevent habituation to the stimuli, the pictures and sounds presented during this practice session were different from the ones used during the actual fear conditioning and extinction tasks. Once the fMRI tasks were completed, a nine-minute structural MRI scan was performed. After the scan, participants were debriefed in a short interview, where they were also asked to identify the actress associated with the scream, to ensure contingency awareness.

2.3.4. Subjective fear rating acquisition

Nervousness ratings were recorded during each presentation of the stimuli (CS + before [potential] apparition of US, and CS−) using a right hand-held button response box developed to allow for a graded range of responses (Current Designs, Philadelphia, PA). Participants were asked to indicate on a five-point Likert scale the degree to which they felt afraid when viewing the actress in the CS + and CS–photos (Are you afraid? 1 = not at all, 5 = extremely).

2.3.5. Skin conductance rates (SCRs) acquisition

The procedures to measure SCRs in the fMRI scanner were developed by Dr. Pierre Rainville of the University of Montreal (Dube et al., 2009). SCRs were recorded during the functional scan using two 10-mm radio-translucent electrodes placed on the plantar surface of the participant's right foot and connected to hardware filters that prevent the cross-contamination of signals (see http://www.biopac.com/mri-compatible-transducer-filtered-cables-specifications for more information). SCRs data were amplified, digitized, and recorded at 1000 Hz using a computerized data acquisition system (MP150-BIOPAC).

2.3.6. fMRI acquisition parameters

Functional and structural MRI data were acquired using echo planar imaging (EPI) sequences with a Siemens TRIO 3-Tesla scanner equipped with a standard head coil. Visual and auditory stimuli were presented on a laptop computer using E-Prime software (PST, Inc., Pittsburgh, PA); images were projected onto a screen at the foot of the scanner while sounds were transmitted via MRI-compatible headphones. The following parameters were used for functional scans: 32 ascending 3.3 mm axial slices covering the entire brain and parallel to the AC-PC plane using a single-shot gradient echo T2* weighting with a TR: 2300 ms, TE: 30 ms, voxel dimensions: 3.8 × 3.8 × 3.3 mm, matrix size: 64 × 64 mm, and field of view (FOV): 24 cm. Volumetric data for spatial normalization was acquired with the following parameters: MP-RAGE sequence with 176 1 mm axial slices, TR: 2300 ms, TE: 2.98 ms, TI: 900 ms, flip angle: 9°, NEX = 1, matrix size of 256 × 256 mm, bandwidth = 240 Hz/Px, and FOV: 256 mm.

2.4. Data analyses

Demographic, psychological, behavioral, physiological and fMRI data analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL). All data were checked for normality of distribution and outliers. Fear ratings and standard fMRI data were log-transformed and SCRs data were square-root-transformed to reach normality.

2.4.1. Descriptive and psychological data

Two-way analyses of variance (ANOVAs) with harsh parenting (high vs. low) and anxiety levels (high vs. low) as between-subjects factors were used to compare groups in terms of current harsh parenting levels and current anxiety levels, as well as age, socio-economic status (SES), verbal IQ, and mean depression levels (CDI). Chi-squares for quantitative measures were used to investigate potential group differences in terms of sex and pubertal status (Tanner stage) of participants. In addition, mean levels and standard deviations were calculated for Tanner stages (ranging from 1 to 5) for descriptive purposes.

2.4.2. SCR preprocessing

Data were preprocessed as follows: 500 ms mean soothing, 1 s delay signal subtraction, and replacement of negative values by 0 (Dube et al., 2009). The extracted area under the differential curve was limited to the first 3 s following cue onset. This was done to prevent contamination with SCRs triggered by motor responses associated with stimulus rating occurring in the last 3 s portion of each stimulus presentation.

2.4.3. SCR intra-subject data analysis

Due to technical issues, the data of 21 participants were lost. Analyses were performed on the remaining 63 participants (66% of the sample). In a first step, the amplitude of the SCRs was standardized within each participant using Z transformations. This was performed to account for the high intra-subject variability of SCRs from one event to the other. We used mean SCRs to both the CS + and CS–events to allow for statistical analysis comparing SCRs to the CS+ > CS- within each group.

2.4.4. fMRI pre-processing

Data were pre-processed and analyzed with SPM8 (SPM8, http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB (Mathworks). For each participant, pre-processing included: slice timing correction, realignment of functional time series, co-registration of functional and anatomical images (trilinear), spatial normalization in Montreal Neurological Institute (MNI) space, spatial smoothing with a 6 mm full-width half-maximum Gaussian smoothing kernel, and high- and low-pass filtering. Images were visually inspected following preprocessing and the overlap between the template and the normalized images was checked. Movement parameters were extracted during the realignment step. Data of participants who moved on average over 3.5 mm in any plane were excluded from analyses (n = 7). In addition, the mean relative scan-to-scan displacement was computed using the Motion Fingerprint software (Wilke, 2012, 2014) and was 0.16 mm (SD = 0.40) for the sample, indicating overall relative low motion. Data were corrected for noise and motion artifacts using the RobustWLS Toolbox (Diedrichsen & Shadmehr, 2005). This toolbox calculates residual mean-square time series during model estimations to estimate the amount of noise for each volume. Instead of excluding volumes with elevated motion, rWLS uses the factor 1/variance to weight the images such that images with higher motion are given smaller weights relative to images with lower or no motion. This approach was privileged over a more conservative one (e.g., completely removing problematic volumes) to prevent excessive data loss, given the pediatric nature of the sample, which may be particularly prone to motion. Derived motion regressors were included in each intra-subject analysis.

2.4.5. fMRI data intra-subject analysis

A two-level hierarchical model using fixed-effects (single subject level) and mixed-effects (group-level) analyses was then conducted. For subject-level analyses, a general linear model (GLM) was used to estimate changes in brain regional responses using 12 regressors: CS + and CS- during habituation phase, CS + unpaired with US and CS- during early (first 7 CS + unpaired and 14 CS-) and late (last 7 CS + unpaired and 14 CS-) conditioning and early (first 7 CS + and CS-) and late (last 7 CS + and CS-) extinction, and US; the 0–3 seconds following unpaired CS + and CS- presentation were also modeled as conditions of no interest, to avoid contamination of baseline due to US expectancy (Dunsmoor & LaBar, 2012; Linnman, Rougemont-Bücking, Beucke, Zeffiro, & Milad, 2011). Contrast images for CS+ > CS- (early and late conditioning together) were estimated separately for each participant.

2.4.6. fMRI group analysis in SPM8

Based on our a priori hypotheses, we used a region of interest (ROI) approach with small volume corrections. Right and left anterior hippocampus ROIs were defined with masks selected from a previous study (Maheu et al., 2010) to focus specifically on the anterior segment of the

structure. Left and right amygdala, insula, and prefrontal/anterior cingulate cortex (encompassing BAs 11, 24, 25, 32 and 47) ROIs were created using the WFU PickAtlas software, version 3.0.5 (http://fmri.wfubmc.edu/cms/software#PickAtlas). A statistical threshold of $p < 0.05$, family-wise error small-volume corrected was used. The average effects of condition were assessed for conditioning in every region. Further, contrast images were entered in two-way ANOVAs with harsh parenting (low vs. high) and anxiety (low vs. high) as between-subjects factors for left and right amygdalae, hippocampi, and insulae. Individual signal change values at the peak voxel coordinates of structures with significant SPM results were extracted, to plot effect directionality and conduct post hoc analyses with SPSS. SPM values were extracted for all conditions of interest (e.g. CS + unpaired with the US during early conditioning vs. baseline, CS- during early conditioning vs. baseline, CS + unpaired with the US during late conditioning vs. baseline, CS- during late conditioning vs. baseline, etc.). A Bonferroni correction was applied to correct for multiple regions of interest. Alpha was set at $p < .008$ (.05/6). Although no significant differences were observed between groups in terms of participants' sex, fMRI data were reanalyzed including sex as a covariate to explore potential sex effects, given numerical (non-significant) differences in the proportion of female participants between the LH/HA group (74%) and the other groups (48%). Additionally, standard fMRI data were re-analyzed excluding three subjects who did not present with significant amygdala activations to CS + vs. CS- during conditioning, and who were also excluded from PPI analyses, to explore potential influences on main results.

2.4.7. SCR, subjective fear ratings, and fMRI group analyses in SPSS

SCR, subjective fear ratings and extracted beta weights were analyzed in distinct ANOVAs. Four-way repeated-measures ANOVAs with harsh parenting (low vs. high) and/or anxiety (low vs. high) as between-subjects factors, and CS-type (CS+, CS−) and time of cue presentation (early vs. late; early: 14 first cues, late: 14 last cues) as within-subjects factors were conducted. Post hoc Tukey group comparisons test set at an alpha level of 0.05 were further performed on significant ANOVA findings. In addition, a chi-square test was performed to assess potential group differences in terms of the number of participants with valid SCRs, given the elevated number of lost data. Finally, we performed a four-way repeated measures ANOVA with harsh parenting (low vs. high) and/or anxiety (low vs. high) as between-subjects factors to assess for potential harsh parenting and anxiety differences in terms of amount of head motion using the mean scan-to-scan displacement.

2.4.8. fMRI exploratory analyses

Separate contrast weights for CS + and CS- during conditioning were computed for each participant and average effects of conditions were estimated for each condition in bilateral amygdalae and hippocampi for exploratory analyses, to look only for significant activations (and not deactivations) in these regions. Mean percentage signal changes at peak activation voxels were plotted for visualization purposes.

2.4.9. Psycho-physiological interactions (PPI) analyses

To look for group differences in fear circuitry connectivity, we performed a psycho-physiological interaction analysis (PPI; Friston et al., 1997) in SPM8 following procedures presented by O'Reilly and colleagues (O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012) and the SPM8 manual (Ashburner et al., 2010). PPIs test whether functional coupling – defined as positive (for positive PPI) or negative (for negative PPI) correlations between a specific region (selected as seed) and other brain regions – changes significantly as a function of task condition (O'Reilly et al., 2012). In the present study, the left amygdala was selected as the seed region, since group differences from standard GLM analyses emerged in this region.

2.4.10. PPI intra-subject analysis

As for standard analyses, a two-level hierarchical model using fixed-effects (single subject level) and mixed-effects (group-level) analyses was conducted. BOLD time-series were first extracted for each participant from voxels within a sphere with a 4 mm radius surrounding the left amygdala activation peak (xyz = −24 −4 −22), where the strongest task effect in group comparisons for contrast CS+ > CS- were observed (O'Reilly et al., 2012). Participants with any significant amygdala activations during conditioning were included in the analyses (n = 81; 96.4% of the sample). For each participant, this time-series was entered as a regressor into a GLM analysis. Amygdala time-course and main effect of task (CS+ > CS-) were extracted and entered as regressors of no interest.

2.4.11. PPI group analyses

To test for harsh parenting and/or anxiety effects or interactions, all PPI individual discrimination contrasts reflecting the positive and negative interactions (PPI regressor) between the psychological (CS + > CS-) and the physiological (amygdala time-course) variables were entered in two-way repeated measures ANOVAs in SPM (one ANOVA for positive connectivity and another for negative connectivity), with harsh parenting (low vs. high) and anxiety (low vs. high) as between-subjects factors. We used an ROI approach with small volume corrections to extract changes in connectivity between the amygdala and other fear circuitry regions. We used the same insula and PFC masks as for standard analyses. A statistical threshold of $p < 0.05$, family-wise error small-volume corrected was used. A Bonferroni correction was applied to correct for multiple regions of interest. Alpha was set at $p < .004$ (.05/12). Individual PPI-related signal change values at the peak voxel coordinates of structures with significant SPM results were extracted, to plot effect directionality and conduct post hoc analyses with SPSS, when needed. As for standard analyses, data were re-analyzed including sex as a covariate to explore potential sex effects.

3. Results

3.1. Demographic and psychological characteristics

There were no main effects of harsh parenting or anxiety or significant interactions for age, SES, IQ, Tanner stage, sex, or depression levels (all *ps* > .05), indicating that groups were equivalent in these areas. Main effects of harsh parenting history were found on current harsh parenting levels, with higher scores found in high vs. low harsh parenting groups ($F(1,80) = 17.56$, $p < .001$, $\eta^2 = .18$). Main effects of anxiety history were found for parent-reported SCARED-R scores ($F(1, 75) = 7.21$, $p = .009$, $\eta^2 = .09$), showing significantly greater current anxiety levels in the high vs. low anxiety groups. These latter findings confirm the validity of the groups based on trajectories of chronic harsh parenting and anxiety levels. Differences were not significant for child-reported SCARED-R scores (p = .362).

3.2. Behavioral and physiological results

Table 2 shows mean subjective ratings and SCRs for each group. Fig. 3 indicates main findings for subjective fear ratings and SCRs.

3.2.1. Subjective fear ratings and contingency awareness

All participants (100%) were contingency-aware. Significant main

Table 2
Mean subjective fear ratings and SCRs (Z-scores) during early and late conditioning for each group.

	Conditioning Phase			
	Early		Late	
	CS+	CS-	CS+	CS-
Ratings, mean (SD)				
LH				
LA	2.00 (1.14)	1.52 (0.68)	1.83 (1.06)	1.16 (0.26)
HA	2.71 (1.25)	2.22 (0.83)	2.51 (1.21)	1.90 (0.79)
HH				
LA	2.27 (1.14)	1.81 (0.85)	2.06 (1.17)	1.53 (0.75)
HA	1.67 (0.61)	1.70 (0.68)	1.56 (0.74)	1.38 (0.56)
SCRs				
LH				
LA	0.20 (0.33)	−0.01 (0.31)	−0.07 (0.28)	−0.11 (0.28)
HA	0.25 (0.23)	−0.05 (0.27)	−0.05 (0.30)	−0.15 (0.22)
HH				
LA	0.23 (0.45)	−0.15 (0.26)	0.02 (0.35)	−0.10 (0.37)
HA	0.03 (0.31)	0.13 (0.28)	−0.02 (0.27)	−0.14 (0.34)

Note. LH = Low harsh parenting; LA = Low anxiety; HA: High anxiety; HH = High harsh parenting.

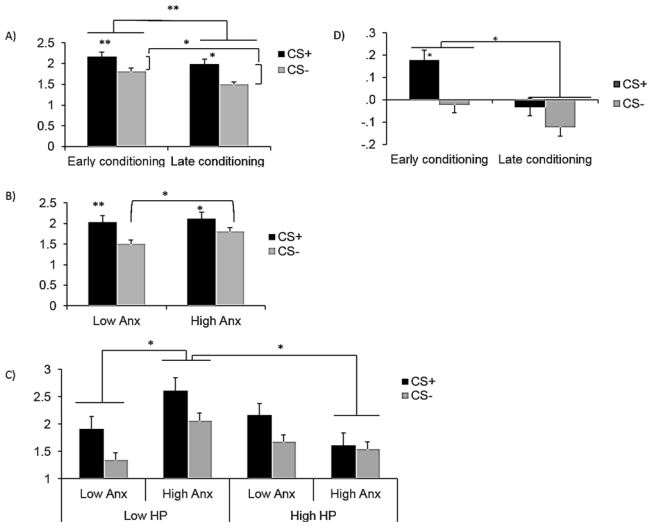

Fig. 3. Subjective fear ratings and SCRs during conditioning. Graph bars represent average ratings; error bars are standard errors. A) CS type x time interaction for fear ratings. B) Anxiety x CS type interaction for fear ratings. C) Harsh parenting x anxiety interaction for fear ratings. Graph bars show the anxiety x CS type interaction in B is driven by the low harsh parenting groups. D) SCRs during conditioning. Graph bars represent average Z-scored SCRs. Main effects of CS type and time of cue presentation.

effects were found for CS type ($F(1, 80) = 30.27$, $p < .001$, $\eta^2 = .28$) and time of cue presentation ($F(1, 80) = 36.27$, $p < .001$, $\eta^2 = .31$). These effects were subsumed by a CS type x time of cue presentation two-way interaction ($F(1,80) = 4.68$, $p = .03$, $\eta^2 = .06$). Post hoc analyses showed that ratings to both CS + and CS- were higher during early vs. late conditioning (both $ps < .001$), and that ratings were higher to CS+ > CS- during both early and late phases (both $ps < .001$), with discrimination conditioning (CS+ > CS-) being greater during late conditioning ($p = .04$). Results also showed a significant anxiety x CS type interaction ($F(1,80) = 4.59$, $p = .04$, $\eta^2 = .05$). Post hoc analyses indicated discrimination conditioning (CS+ > CS-) was acquired in both anxiety groups (respectively $p < .001$ in low anxiety and $p = .02$ in high anxiety), and higher ratings to CS- were observed in high relative to low anxiety ($p = .03$). Finally, a significant harsh parenting x anxiety interaction was found ($F(1, 80) = 8.19$, $p = .005$, $\eta^2 = .09$). Post hoc tests indicated that in the low harsh parenting groups, higher anxiety levels (LH/HA) were associated with greater fear ratings to both CSs ($p = .005$) relative to low anxiety (LH/LA), while no differences were observed between high (HH/HA) and low anxiety (LH/LA) in the high harsh parenting groups ($p = .27$). Moreover, subjective fear ratings were significantly higher in LH/HA group relative to the HH/HA group ($p = .006$).

Table 3
Peak voxels for contrast CS + vs. CS- during conditioning.

Region	Cluster size (voxels)	MNI coordinates			F	FWE corrected p
		x	y	z		
Average effect of condition						
Left amygdala	60	−26	−6	−18	26.61	< .0001
Right amygdala	53	30	−4	−28	21.73	< .001
Left anterior hippocampus	209	−26	−14	−18	41.99	< .00001
Right anterior hippocampus	253	34	−18	−18	47.57	< .000001
Left insula	462	−36	−6	12	17.94	.01
Right insula	963	46	−16	12	31.10	< .001
Left BA24	43	−6	32	−6	17.75	< .01
Right BA 24	46	8	24	−6	28.63	< . 001
Left BA 32	301	−10	38	−10	41.31	< .00001
Right BA 32	278	6	44	−10	36.73	< .0001
Main effect of harsh parenting						
Left amygdala	33	−24	−4	−22	14.85	.005
Left anterior hippocampus	95	−26	−8	−20	15.79	.005
Right anterior hippocampus	36	36	−14	−16	11.52	.03

Cluster size at uncorrected p < .005. Coordinates at most significant peak for each region.

3.2.2. SCRs

There was no significant difference in the number of participants with valid SCRs ($\chi^2(3) = 3.89$, $p = .274$), suggesting data loss impacted the groups evenly (see Table 1 for numbers per group). Main effects of CS type and time of cue presentation were found, with greater SCRs observed for CS+ > CS- ($F(1, 58) = 11.68$, $p = .001$, $\eta^2 = .17$), and during early vs. late conditioning ($F(1,58) = 12.26$, $p = .001$, $\eta^2 = .18$). No other significant main effects or interactions were found (all $ps > .05$).

3.4. fMRI results

3.4.1. Standard GLM analyses

3.4.1.1. Overall conditioning effects. The main effects of condition showed significant activations to CS+ > CS- in bilateral amygdalae, hippocampi, insulae, and sgACC/vmPFC (BAs 24 and 32) across participants (see Table 3 for exact coordinates and statistics for each ROI).

3.4.1.2. Group comparisons. SPM group comparisons of activations to CS + vs. CS- revealed a significant main effect of harsh parenting in the left amygdala and in the left and right anterior hippocampi (see Table 3 for exact coordinates and statistics for each ROI and Fig. 4 for activation map). Only activations in the left amygdala and left anterior

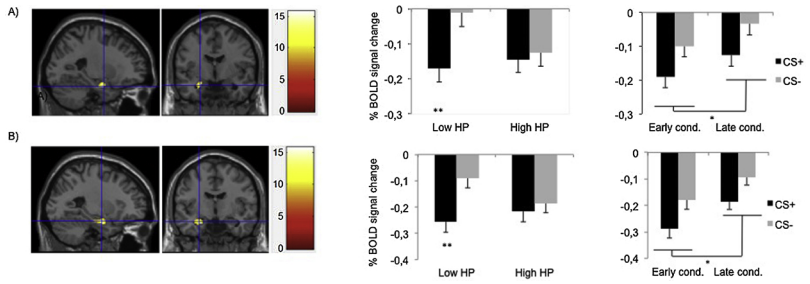

Fig. 4. Amygdala and anterior hippocampus activation to CS + > CS- during conditioning. Images presented at an uncorrected $p < .005$ threshold. Graph bars represent mean % BOLD signal change. A) Greater activation to CS+ vs. CS- in high vs. low harsh parenting in left amygdala (xyz = −24 −4 −22), and in B) left anterior hippocampus (xyz = −26 −8 −20).

Table 4
Exploratory analyses: peak activation voxels to CS + vs. baseline and CS- vs. baseline in amygdalae and hippocampus during conditioning (included in the SI section).

Region	CS + vs. baseline						CS- vs. baseline					
	Cluster size (voxels)	MNI coordinates			F	FWE corrected p	Cluster size (voxels)	MNI coordinates			F	FWE corrected p
		x	y	z				x	y	z		
Average effect of condition												
Left amygdala	37	−24	−8	−16	48.67	< .000001	22	−24	0	−12	60.33	< .000001
	11	−24	0	−12	30.79	< .0001	11	−24	−8	−16	14.94	< .01
Right amygdala	72	30	−4	−20	44.41	< .000001	23	32	−4	−20	20.23	< .01
Left ant. hippo.	195	−24	−12	−18	104.02	< .000001	152	−24	−12	−18	46.26	< .000001
Right ant. hippo.	256	24	−12	−18	83.40	< .000001	190	24	−12	−18	40.48	< .00001

Cluster size at uncorrected p < .005.

hippocampus remained significant after Bonferroni correction. Removing the three subjects without amygdala activation did not significantly alter results. Therefore, these subjects were included in further analyses. Follow-up ANOVAs using SPSS on the signal changes in the identified peak suprathreshold voxels revealed similar results for the two significant structures. First, a main effect of time was found, with greater activations (or less deactivations) during late vs. early conditioning (respectively ($F(1, 82) = 4.92$, $p = .04$, $\eta^2 = .05$ for left amygdala, $F(1,82) = 10.53$, $p = .002$, $\eta^2 = .11$ for left anterior hippocampus). A main effect of CS type was found ($F(1, 82) = 23.38$, $p < .001$, $\eta^2 = .22$ for left amygdala, $F(1,82) = 31.60$, $p < .001$, $\eta^2 = .28$ for left anterior hippocampus), as well as a significant CS type x harsh parenting interaction ($F(1,82) = 13.99$, $p < .001$, $\eta^2 = .15$ for left amygdala, $F(1,82) = 14.41$, $p < .001$, $\eta^2 = .15$ for left anterior hippocampus). Post hoc analyses revealed significant CS+ > CS- differences in low harsh parenting only. Specifically, in the low harsh parenting groups, greater deactivations were observed for CS + relative to CS- for all structures (all *ps* < .001) while no CS+/CS- differences appeared in the high harsh parenting groups (all *ps* > .1). Adding sex as a covariate did not significantly change these results.

Regarding head motion, no significant effects of harsh parenting or anxiety, or significant interactions between the two factors, were found in terms of mean scan-to-scan displacement ($F(3) = 1.07$, $p = .367$, see Table 1 for values per group), suggesting groups were equivalent in terms of head motion.

3.4.1.3. Exploratory analyses on limbic regions. Average effects of conditions for CS + and CS- analyzed separately showed significant overall activations in bilateral amygdalae and hippocampi for both stimuli relative to baseline (all *ps* < .01 FWE-corrected; see Table 4 for exact coordinates and statistics for each ROI and Fig. 5 for activation map), suggesting these areas were positively activated during conditioning, despite the deactivations observed in group comparisons.

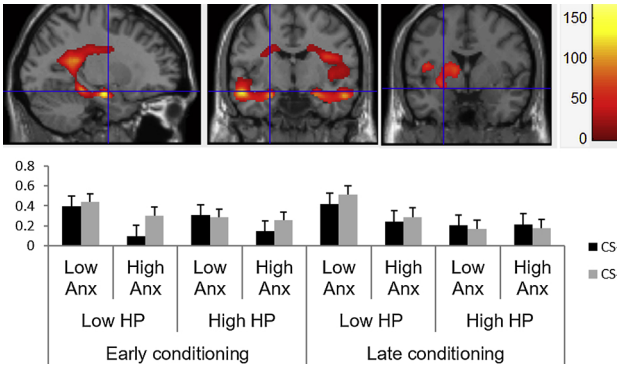

Fig. 5. Average effects of CS+ > baseline and CS- > baseline (all subjects together) during conditioning in left and right amygdalae. Images presented at an uncorrected p < .001 threshold. Graph bars represent mean % signal change to CS + and CS- in left amygdala (xyz = −24 0–12).

3.4.2. PPI GLM analyses

3.4.2.1. Positive connectivity. SPM PPI analyses revealed main effects of harsh parenting for positive PPIs between left amygdala and left and right insula (see Table 5 for exact statistics for each ROI and Fig. 6 for activation map). Extracted beta weights show greater positive connectivity between left amygdala and bilateral insula to CS+ > CS- in low vs. high harsh parenting.

Table 5
Peak voxels for groups comparisons for CS + vs. CS- positive and negative PPI contrasts with left amygdala as seed region during conditioning.

Region	Positive PPI						Negative PPI					
	Cluster size (voxels)	MNI coordinates			F	FWE corrected p	Cluster size (voxels)	MNI coordinates			F	FWE corrected p
		x	y	z				x	y	z		
Main effect of HP												
Left insula	110	−38	6	14	16.79	.02	47	−38	6	14	14.40	.04
Right insula	102	42	4	14	17.86	.02	83	42	0	14	17.05	.02
HP* Anxiety interaction												
Right BA 24	31	6	32	4	14.40	.04						
							Cluster size at uncorrected p < .005					

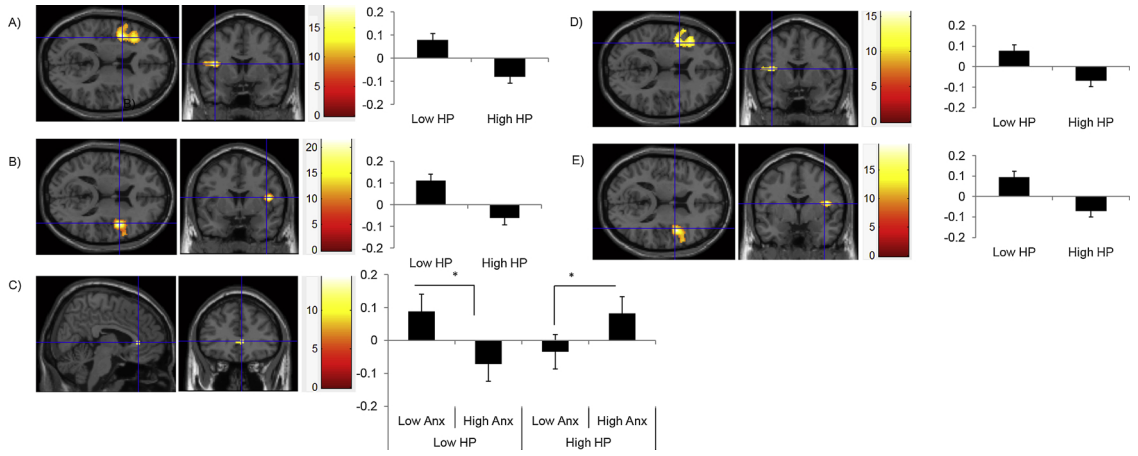

Fig. 6. Positive (A, B, C) and negative (D, E) CS+ > CS- PPIs with left amygdala (xyz= −24 −4 −22) as seed region during conditioning. Images presented at an uncorrected p < .005 threshold. Graph bars represent mean % BOLD signal change. Increased positive and negative connectivity with amygdala in left (A; xyz = −38 6 14 and D; xyz = −38 6 14) and right (B; xyz = 42 4 14 and E; xyz = 42 0 14) insula in low vs. high harsh parenting. C) Harsh parenting x anxiety interaction on positive connectivity with right BA 24 (xyz = 6 32 4).

A harsh parenting x anxiety interaction was found for positive amygdala- rostral ACC (rACC/ BA 24) connectivity. Subsequent ANOVAs performed on SPSS with extracted beta weights confirmed the harsh parenting x anxiety interaction ($F(1,77) = 13.98$, $p < .001$, $\eta^2 = .15$). Post hoc analyses showed that in low harsh parenting, high anxiety levels (LH/HA) were associated with lower amygdala-rACC positive coupling to CS+ > CS-, relative to low anxiety levels (LH/LA) ($p = .003$). In high harsh parenting, however, high anxiety (HH/HA) was associated with greater amygdala-rACC positive connectivity relative to low anxiety (HH/LA) ($p = .03$).

3.4.2.2. Negative connectivity. SPM analyses also revealed main effects of harsh parenting for negative PPIs between the left amygdala and left and right insula (see Table 5 for exact statistics for each ROI and Fig. 6 for activation map). Extracted beta weights showed increased negative coupling between left amygdala and bilateral insula to CS+ > CS- in low harsh parenting relative to high harsh parenting.

As for standard analyses, adding sex as a covariate did not significantly change results of PPI analyses. After Bonferroni correction, connectivity results were no longer significant. However, given the exploratory nature of the study, we interpreted all results that were significant before applying the correction for multiple comparisons.

4. Discussion

To our knowledge, this is the first study to investigate the separate and joint associations of harsh parenting and anxiety with fear conditioning in psychiatrically healthy youths. At the neural level, differences related to harsh parenting were observed in terms of medial temporal lobe activations and connectivity with the insula. Interestingly, harsh parenting and anxiety interactions were also found both at the behavioral level and with regard to amygdala-rACC coupling.

Overall, subjective fear ratings, SCRs, and medial temporal lobe activations to CS+ > CS- suggest discrimination conditioning was effectively acquired across participants. In terms of time effects, behavioral and physiological data show that although CS+ > CS- differences were established from the beginning of the procedure, and despite a general habituation to both CSs, the threat-safety conscious differentiation increased with increased exposure to both CSs. In terms of medial temporal lobe responses, higher overall activations to both CSs were found during late relative to early conditioning.

As expected, greater medial temporal lobe (left amygdala and anterior hippocampus) activations to CS+ > CS- were found in high relative to low harsh parenting. This is consistent with previous studies suggesting greater sensitivity to threat in adversely-reared individuals and in persons carrying other risks for anxiety (Bremner et al., 2005; Craske et al., 2008; Indovina et al., 2011; Maheu et al., 2010; Pejic, Hermann, Vaitl, & Stark, 2013), which probably reflects the development of hypervigilance mechanisms that prepare them to face potential dangers from a threatening environment (e.g., McCrory et al., 2017).

Of note, these differences were due to greater deactivations to the CS + relative to the CS- in low harsh parenting. Moreover, beta weights indicate amygdala and anterior hippocampus deactivations to the CS + and CS- in both groups as soon as the early conditioning phase. This may reflect rapid habituation processes, as previously demonstrated in the literature (Buchel, Morris, Dolan, & Friston, 1998). Medial temporal lobe deactivations could also be due to higher baseline activation in our participants, owing to increased arousal levels triggered by the scanning procedure. This may explain the reduction in

deactivations observed over time, as participants became more familiar with the machine and procedures. Nevertheless, follow-up analyses show that other amygdala and anterior hippocampus clusters were significantly activated across groups to CS+ (and CS-) relative to baseline. However, harsh parenting differences were only observed in deactivated clusters. Deactivations in some amygdala subregions in our healthy youth sample may reflect inhibitory inputs from other amygdala regions (Duvarci & Pare, 2014) and/or other higher order regions such as the sgACC (Marek et al., 2013), which was also significantly activated across groups. In the low harsh parenting groups, these inputs may target specifically the threat-signaling stimulus (i.e. CS+), while this may not be the case in the high harsh parenting groups. Hence, a harsh parenting history may predispose youths for psychopathology not only because of a hypersensitivity to threat but also due to a less targeted suppression of fear responses and/or a less efficient modulation of attention to threat.

Amygdala and hippocampus deactivations during threat processing have been previously reported in healthy individuals (e.g., Petrovic, Carlsson, Petersson, Hansson, & Ingvar, 2004), and have been suggested to reflect mechanisms of attenuation of stress responses (Petrovic et al., 2004). On the other hand, a recent study by Puetz and colleagues (Puetz et al., 2016) found amygdala hypoactivation to social threat cues in maltreated children and adolescents relative to controls. In parallel, hippocampus hypoactivation has been linked to increased anxiety symptoms during stress-evoking situations in trauma-exposed youths, while no such relation was found in control participants (Elsey et al., 2015). The authors interpreted this as a blunting of stress responses in trauma-exposed youths, due to repetitive exposure to stress (Elsey et al., 2015; Puetz et al., 2016). Therefore, decreased medial temporal lobe function may reflect adaptive responses to threat in the low harsh parenting participants, while it may be maladaptive to the high harsh parenting youths, perhaps reflecting deficits in emotion regulation.

Lower functional connectivity between left amygdala and bilateral insula in response to CS+ > CS- was also found in high relative to low harsh parenting. Previous studies demonstrated the insula's role in the perception of interoceptive states (Simmons et al., 2013), as well as in the integration of interoceptive information with perceptual and high-level cognitive representations, through heavy connections with somatosensory regions and limbic system structures such as the amygdala, PFC, and ACC regions (Simmons et al., 2013). This may help evaluate the self-relevance of internal and external information, which may, in turn, be critical for producing appropriate affective responses (Simmons et al., 2013). Decreased amygdala-insula functional connectivity has indeed been reported in anxious and depressed individuals and in people with histories of early adversity (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009; Perlman et al., 2012; Van der Werff et al., 2013). It has been suggested that these alterations reflect the impaired integration of emotions generated by the amygdala into conscious processing by the insula (Perlman et al., 2012). It may be that reduced amygdala-insula connectivity in the high harsh parenting groups indirectly impairs proper regulation of amygdala responses to CS + through a poor awareness of emotional states, which therefore cannot be effectively communicated to emotion-regulation regions. However, it is important to recall that these results are only exploratory and did not survive correction for multiple comparisons. Further studies are needed to corroborate our findings.

No further differences were observed between high and low anxiety groups, independently of harsh parenting levels, in terms of discrimination conditioning, both at the neural and at the behavioral/physiological levels. However, increased fear ratings to CS- were found in high relative to low anxiety, despite equivalent levels of discrimination conditioning. These findings are consistent with findings in clinically anxious individuals, suggesting a generalization of fear responses to the safety signal (i.e. CS-) (Lissek, 2012; Lissek et al., 2005). This, however, seems to be present only in a context of low adversity. Indeed, anxiety differences seemed to be driven by the low harsh parenting groups, suggesting that harsh parenting may have a modulatory influence on anxiety. Moreover, high anxiety was related to greater overall fear ratings of both CS + and CS- relative to low anxiety, but only in the low harsh parenting groups. This may suggest that a history of harsh parenting may lead to a unique anxious phenotype, which is separate from anxious manifestations of other sources.

Along the same lines, functional connectivity analyses showed the direction of anxiety differences varied according to harsh parenting contexts. In low harsh parenting groups, high relative to low trait anxiety levels were associated with lower amygdala-rostral ACC (rACC) positive functional connectivity, while in a context of high harsh parenting, high anxiety levels were associated with higher amygdala-rACC connectivity. These results could be explained, at least in part, by a greater – although non-significant—proportion of female participants in the LH/HA group (74%) relative to the other groups (48%). Indeed, previous research suggested reduced amygdala-ACC connectivity in females relative to males exposed to early life trauma (Helpman et al., 2017). However, controlling for child sex did not significantly alter results, suggesting other factors may better explain findings. Reduced amygdala-mPFC/rACC connectivity has been reported in clinically anxious adults (Etkin et al., 2010), and in individuals with a history of childhood maltreatment, in whom it was associated with increased anxiety symptoms (Birn et al., 2014; Herringa et al., 2013; Thomason et al., 2015). Hence, reduced connectivity in youths of the LH/HA group may predispose them towards later anxiety disorders. However, the greater connectivity observed in the HH/HA group is quite unexpected. An alternative explanation may be that higher positive amygdala-rACC connectivity is not necessarily beneficial, especially in a context of early and chronic adversity. In maltreated children and adolescents, negative amygdala-mPFC/rACC connectivity has been associated with lower anxiety symptoms, possibly reflecting developmental adaptation mechanisms that aim to regulate a hyper-responsive amygdala (Gee et al., 2013). By contrast, positive amygdala-mPFC connectivity has been associated with immature neural function and greater emotional reactivity (Gee et al., 2013). As a matter of fact, Kalisch and Gerlicher (2014)) highlighted the role of rACC in the cognitive dimensions of anxiety, including conscious threat appraisal, worrying and catastrophizing. Lower positive amygdala-rACC functional connectivity in the two groups carrying only one risk factor, either harsh parenting or high trait anxiety (i.e. HH/LA and LH/HA), may thus represent a protective mechanism that may prevent later psychopathology development. Lower levels were not observed in the HH/HA group, which presents with a double risk for anxiety disorders (i.e. high trait anxiety and a history of early adversity); this may put youths in this group at greater risk of psychopathology. A final potential explanation is that greater functional connectivity may have a different interpretation according to harsh parenting history, because of the observed differences in amygdala function. Hence, in a context of a well-regulated amygdala, as encountered in low harsh parenting, greater, positive amygdala-rACC connectivity is associated with low anxiety symptoms, whereas in high harsh parenting, where the amygdala is not properly regulated, greater connectivity with rACC may lead to a greater propensity to worries and catastrophizing, and thus be associated with greater trait anxiety levels. Again, however, one must remember the preliminary nature of these results, which were no longer significant after Bonferroni correction. Future studies with larger samples are needed in order to confirm these findings.

Taken together, although preliminary, the present findings suggest specific associations of harsh parenting and trait anxiety with fear circuitry function, as well as interactions between the two risk factors. Hence, a history of chronic elevated harsh parenting levels is associated with lower medial temporal lobe regulation, and with lower amygdala-insula functional connectivity during threat signals processing. In terms of amygdala-rostral ACC connectivity, however, anxiety differences varied according to harsh parenting levels. This highlights the need to consider both risk factors when planning interventions, as the

underlying neural mechanisms may differ according to early adversity and anxiety histories. Further studies are needed to corroborate these findings and to assess potential associations between the distinct connectivity patterns identified here and later psychopathology as well as response to treatment.

This study has several limitations. First, anxiety and harsh parenting differences were observed during differential conditioning for fear ratings and brain activations, but not for SCR measures. Discrepancies between SCRs and rating measures have been previously reported (e.g., Chauret et al., 2014; Pejic et al., 2013) and may be explained by distinct underlying neural mechanisms. Physiological responses are unconscious and automatic responses allowing for rapid processing of information and triggered mainly by the amygdala, whereas fear ratings represent conscious cognitive responses relying on prefrontal regions (LeDoux, 2014). In addition, an absence of SCR differences between anxious and non-anxious participants in a context of behavioral and/or brain activation differences is not uncommon (e.g., Tzschoppe et al., 2014). Alternatively, the absence of effects observed on SCR measures may be due to a lack of statistical power as SCR data was missing for 34% of the sample. Second, as previously mentioned, results from connectivity analyses did not survive corrections for multiple comparisons. Hence, findings must be interpreted with caution given their preliminary nature. Replication studies are needed to confirm the validity of our results.

Third, harsh parenting practices were self-reported, so mothers may have under-evaluated their levels of harsh parenting. Nevertheless, previous studies have suggested self-report to be a valid and useful measure for etiologic research (Windham et al., 2004), and care was taken to minimize social desirability by intercalating questions on harsh parenting with other measures including positive parenting. Moreover, the harsh parenting questions employed in the previous study have been extensively used (e.g., Battaglia et al., 2016; Galéra et al., 2014; Pierce et al., 2010), and the levels of harsh parenting observed were consistent with those of previous research (e.g., Lansford et al., 2009). Additionally, significant negative correlations between the harsh parenting items used in the present study and measures of the Maternal Q-Sort (Moran, Pederson, & Bento, 2009), a clinician-rated measure of maternal sensitivity, were previously observed (Tarabulsy, 2012), supporting the adequate validity of the measure. The fact that harsh parenting was measured in mothers only is another limitation of this study. However, previous studies in the same cohorts reported paternal and maternal harsh parenting levels to be positively correlated, suggesting that similar patterns would most probably have been observed in our participants' fathers (Guimond et al., 2012). In addition, although parent-rated current anxiety levels confirmed the validity of our four groups, child-rated anxiety levels were not statistically different from one group to the other. Differences between child and parent reports are not uncommon for mental health measures (e.g., Varni et al., 2015), and underline the need for combining both measures when assessing anxiety in youths. Thus, future studies should include child-rated anxiety measures throughout development, within the limits of the child's capacities.

Another important limitation is that we did not investigate parental history of anxiety or other psychiatric disorders. Genetic background influences anxiety disorders development and fear circuitry function and may interact with early adversity factors (Casey et al., 2011; Redlich et al., 2015); hence, we can not rule out the possibility that our results may have been led in part by genetic factors. Although differences were not statistically significant, the proportion of females was numerically higher in the LH/HA group relative to the other groups, which may have influenced results, as sex differences have been previously observed in fear conditioning responses (e.g., Chauret et al., 2014; Milad et al., 2006) and brain connectivity (Helpman et al., 2017). However, controlling for child sex did not significantly alter fMRI results. Future studies should include an equivalent number of males and females across groups as well as measures of familial psychopathology background and paternal harsh parenting levels.

Finally, one must keep in mind that all of our participants were psychiatrically healthy and showed only subclinical levels of trait anxiety. Hence, despite being at greater risks of psychopathology relative to their low anxiety counterparts, youths in the high anxiety groups were still considered resilient at the moment of the study. Indeed, some of these youths may never develop an anxiety disorder, which limits interpretations regarding potential relations with later psychopathology. However, trait anxiety has been identified as a key feature of anxiety disorders that is associated with both the etiology and the course of the disorder (Kampman et al., 2017), suggesting a greater number of youths in the high relative to low anxiety groups will likely develop clinical anxiety levels in the future. Longitudinal studies are needed to follow youths further and into adulthood.

Regardless of these limitations, this study sheds light for the first time on the individual and joint associations of harsh parenting and anxiety with fear processing in youth. The strength of this study lies in the repeated assessments of harsh parenting and anxiety levels over a 10-year period in a large and representative population-based cohort. This work partially replicates previous findings in individuals with histories of anxiety and/or childhood adversity, extends knowledge to youth undergoing a "milder" but unfortunately still widespread form of adversity, and provides new insights into neural mechanisms underlying the risks of and resiliency to future psychopathology.

Declaration of interest

None.

Acknowledgements

This research was supported by grants from the Canadian Institutes for Health Research (CIHR; #MOP-97983, # MOP-44072 and #HDF-70335), the Quebec Government's Ministry of Health, the Fonds Québécois de la Recherche sur la Société et la Culture (FQRSC), Canada's Social Science and Humanities Research Council (SSHRC), CHU Ste-Justine's Research Center, the University of Montreal, and Université Laval. FSM is a CIHR New Investigator and Fonds de recherche en santé du Québec (FRSQ) Junior 1 Awardee. VLB received PhD fellowships from the FRSQ and CIHR. Michel Boivin is supported by the Canada Research Chair Program. The funding sources had no involvement in study design, collection, analysis, and interpretation of data, writing of the manuscript, and decision to submit the manuscript for publication. We are grateful to the parents and children of the Québec Longitudinal Study of Child Development (QLSCD). We thank the Institut de la Statistique du Québec and their partners, and the Research Unit on Children's Psychosocial Maladjustment (RUCPM) staff for data collection and management. We thank Charles-Édouard Giguère and Hélène Paradis from the RUCPM for their help with data management and analysis.

References

Achenbach, T. M. (1991). *Child behavior checklist.* Burlington, VT: U. o. V. Department of Psychiatry.

Ashburner, J., Barnes, G., Chen, C.-C., Daunizeau, J., Flandin, G., Friston, K., ... Phillips, C. (2010). *SPM8 manual*.

Battaglia, M., Touchette, É., Garon-Carrier, G., Dionne, G., Côté, S. M., Vitaro, F., ... Boivin, M. (2016). Distinct trajectories of separation anxiety in the preschool years: Persistence at school entry and early-life associated factors. *Journal of Child Psychology and Psychiatry, 57*(1), 39–46. https://doi.org/10.1111/jcpp.12424.

Birmaher, B., Khetarpal, S., Brent, D., Cully, M., Balach, L., Kaufman, J., ... Neer, S. M. (1997). The screen for child anxiety related emotional disorders (SCARED): Scale construction and psychometric characteristics. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(4), 545–553. https://doi.org/10.1097/00004583-199704000-00018.

Birn, R. M., Patriat, R., Phillips, M. L., Germain, A., & Herringa, R. J. (2014). Childhood maltreatment and combat posttraumatic stress differentially predict fear-related fronto-subcortical connectivity. *Depression and Anxiety, 31*(10), 880–892. https://doi.

org/10.1002/da.22291.
Boivin, M., Perusse, D., Dionne, G., Saysset, V., Zoccolillo, M., Tarabulsy, G. M., ... Tremblay, R. E. (2005). The genetic-environmental etiology of parents' perceptions and self-assessed behaviours toward their 5-month-old infants in a large twin and singleton sample. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 46*(6), 612–630. https://doi.org/10.1111/j.1469-7610.2004.00375.x JCPP375 [pii].
Boyle, M. H., Jenkins, J. M., Georgiades, K., Cairney, J., Duku, E., & Racine, Y. (2004). Differential-maternal parenting behavior: Estimating within- and between-family effects on children. *Child Development, 75*(5), 1457–1476. https://doi.org/10.1111/j.1467-8624.2004.00751.x CDEV751 [pii].
Bremner, J. D., Vermetten, E., Schmahl, C., Vaccarino, V., Vythilingam, M., Afzal, N., ... Charney, D. S. (2005). Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. *Psychological Medicine, 35*(6), 791–806.
Buchel, C., Morris, J., Dolan, R. J., & Friston, K. J. (1998). Brain systems mediating aversive conditioning: An event-related fMRI study. *Neuron, 20*(5), 947–957.
Casey, B., Ruberry, E. J., Libby, V., Glatt, C. E., Hare, T., Soliman, F., ... Tottenham, N. (2011). Transitional and translational studies of risk for anxiety. *Depression and Anxiety, 28*(1), 18–28.
Chauret, M., La Buissonniere-Ariza, V., Lamoureux Tremblay, V., Suffren, S., Servonnet, A., Pine, D. S., ... Maheu, F. S. (2014). The conditioning and extinction of fear in youths: What's sex got to do with it? *Biological Psychology, 100*, 97–105. https://doi.org/10.1016/j.biopsycho.2014.06.001.
Clément, M.-E., Bernèche, F., Fontaine, C., & Chamberland, C. (2012). Les attitudes parentales et les pratiques familiales. In G. Tardif (Ed.). *La violence familiale dans la vie des enfants du québec*. Montreal, QC, Canada: Institut de la Statistique du Québec.
Cote, S. M., Boivin, M., Liu, X., Nagin, D. S., Zoccolillo, M., & Tremblay, R. E. (2009). Depression and anxiety symptoms: Onset, developmental course and risk factors during early childhood. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 50*(10), 1201–1208. https://doi.org/10.1111/j.1469-7610.2009.02099.x.
Craske, M. G., Waters, A. M., Lindsey Bergman, R., Naliboff, B., Lipp, O. V., Negoro, H., ... Ornitz, E. M. (2008). Is aversive learning a marker of risk for anxiety disorders in children? *Behaviour Research and Therapy, 46*(8), 954–967. https://doi.org/10.1016/j.brat.2008.04.011.
Diedrichsen, J., & Shadmehr, R. (2005). Detecting and adjusting for artifacts in fMRI time series data. *Neuroimage, 27*(3), 624–634.
Dube, A.-A., Duquette, M., Roy, M., Lepore, F., Duncan, G., & Rainville, P. (2009). Brain activity associated with the electrodermal reactivity to acute heat pain. *Neuroimage, 45*(1), 169–180.
Dunn, L. M., & Dunn, L. (1981). *Peabody picture vocabulary test-revised (PPVT): Manual for forms l and m.* Circle Pines, MN: American Guidance Service.
Dunn, L. M., Theriault-Whalen, C. M., & Dunn, L. M. (1993). *Échelle de Vocabulaire en Images Peabody. Adaptation française du Peabody Picture Vocabulary Test-Revised. Manuel pour les formes A et B.* Toronto, Ontario: CANADA: PSYCAN.
Dunsmoor, J. E., & LaBar, K. S. (2012). Brain activity associated with omission of an aversive event reveals the effects of fear learning and generalization. *Neurobiology of Learning and Memory, 97*(3), 301–312.
Duvarci, S., & Pare, D. (2014). Amygdala microcircuits controlling learned fear. *Neuron, 82*(5), 966–980.
Elsey, J., Coates, A., Lacadie, C. M., McCrory, E. J., Sinha, R., Mayes, L. C., ... Potenza, M. N. (2015). Childhood trauma and neural responses to personalized stress, favorite-food and neutral-relaxing cues in adolescents. *Neuropsychopharmacology, 40*(7), 1580–1589.
Etkin, A., Prater, K. E., Hoeft, F., Menon, V., & Schatzberg, A. F. (2010). Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *The American Journal of Psychiatry, 167*(5), 545–554. https://doi.org/10.1176/appi.ajp.2009.09070931.
Etkin, A., Prater, K. E., Schatzberg, A. F., Menon, V., & Greicius, M. D. (2009). Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Archives of General Psychiatry, 66*(12), 1361–1372.
Friston, K., Buechel, C., Fink, G., Morris, J., Rolls, E., & Dolan, R. (1997). Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage, 6*(3), 218–229.
Galéra, C., Pingault, J.-B., Michel, G., Bouvard, M.-P., Melchior, M., Falissard, B., ... Côté, S. M. (2014). Clinical and social factors associated with attention-deficit hyperactivity disorder medication use: Population-based longitudinal study. *The British Journal of Psychiatry, 205*(4), 291–297. https://doi.org/10.1192/bjp.bp.113.141952.
Gee, D. G., Gabard-Durnam, L. J., Flannery, J., Goff, B., Humphreys, K. L., Telzer, E. H., ... Tottenham, N. (2013). Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. *Proceedings of the National Academy of Sciences of the United States of America, 110*(39), 15638–15643. https://doi.org/10.1073/pnas.1307893110.
Gershoff, E. T. (2008). *Report on physical punishment in the United States: What research tells us about its effects on children*Columbus, OH.
Gidron, Y. (2013). Trait anxiety. In T. J. R. e, & M. D. Gellman (Eds.). *Encyclopedia of behavioral medicine*. New York, NY: Springer.
Guimond, F.-A., Brendgen, M., Forget-Dubois, N., Dionne, G., Vitaro, F., Tremblay, R. E., ... Boivin, M. (2012). Associations of mother's and father's parenting practices with children's observed social reticence in a competitive situation: A monozygotic twin difference study. *Journal of Abnormal Child Psychology, 40*(3), 391–402.
Hardee, J. E., Benson, B. E., Bar-Haim, Y., Mogg, K., Bradley, B. P., Chen, G., ... Perez-Edgar, K. (2013). Patterns of neural connectivity during an attention bias task moderate associations between early childhood temperament and internalizing symptoms in young adulthood. *Biological Psychiatry, 74*(4), 273–279. https://doi.org/10.1016/j.biopsych.2013.01.036.
Hart, H., & Rubia, K. (2012). Neuroimaging of child abuse: A critical review. *Frontiers in Human Neuroscience, 6*, 52. https://doi.org/10.3389/fnhum.2012.00052.
Hein, T. C., & Monk, C. S. (2017). Research Review: Neural response to threat in children, adolescents, and adults after child maltreatment – A quantitative meta-analysis. *Journal of Child Psychology and Psychiatry, 58*(3), 222–230. https://doi.org/10.1111/jcpp.12651.
Helpman, L., Zhu, X., Suarez-Jimenez, B., Lazarov, A., Monk, C., & Neria, Y. (2017). Sex differences in trauma-related psychopathology: A critical review of neuroimaging literature (2014-2017). *Current Psychiatry Reports, 19*(12), 104. https://doi.org/10.1007/s11920-017-0854-y.
Herringa, R. J., Birn, R. M., Ruttle, P. L., Burghy, C. A., Stodola, D. E., Davidson, R. J., ... Essex, M. J. (2013). Childhood maltreatment is associated with altered fear circuitry and increased internalizing symptoms by late adolescence. *Proceedings of the National Academy of Sciences of the United States of America, 110*(47), 19119–19124. https://doi.org/10.1073/pnas.1310766110.
Hofmann, S. G., Ellard, K. K., & Siegle, G. J. (2012). Neurobiological correlates of cognitions in fear and anxiety: A cognitive-neurobiological information-processing model. *Cognition & Emotion, 26*(2), 282–299. https://doi.org/10.1080/02699931.2011.579414.
Hollingshead, A. B. (1973). *Four-factor index of social status.* New Haven: Yale University Press.
Indovina, I., Robbins, T. W., Nunez-Elizalde, A. O., Dunn, B. D., & Bishop, S. J. (2011). Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron, 69*(3), 563–571. https://doi.org/10.1016/j.neuron.2010.12.034.
Jetté, M., & Des Groseilliers, L. (2000). *Survey description and methodology of the Longitudinal Study of Child Development in Québec (ÉLDEQ 1998-2002) Québec.* Canada: Institut de la statistique du Québec.
Jetté, M., Desrosiers, H., & Tremblay, R. E. (1998). *Santé Québec "In 2001… I'll be 5 years old" - Survey of 5 months old infants: Preliminary report from the Québec Longitudinal Study of Childhood Development [«En 2001… J'aurai 5 ans !», Enquête auprès des bébés de 5 mois, Rapport préliminaire de l'Étude longitudinale du développement des enfants du Québec (ÉLDEQ).]*Montréal, QC, CANADA: Ministère de la Santé et des Services sociaux, Gouvernement du Québec.
Jovanovic, T., Nylocks, K. M., Gamwell, K. L., Smith, A., Davis, T. A., Norrholm, S. D., ... Bradley, B. (2014). Development of fear acquisition and extinction in children: Effects of age and anxiety. *Neurobiology of Learning and Memory, 113*, 135–142. https://doi.org/10.1016/j.nlm.2013.10.016.
Kalisch, R., & Gerlicher, A. M. (2014). Making a mountain out of a molehill: On the role of the rostral dorsal anterior cingulate and dorsomedial prefrontal cortex in conscious threat appraisal, catastrophizing, and worrying. *Neuroscience and Biobehavioral Reviews, 42*, 1–8.
Kampman, O., Viikki, M., & Leinonen, E. (2017). Anxiety disorders and temperament-an update review. *Current Psychiatry Reports, 19*(5), 27. https://doi.org/10.1007/s11920-017-0779-5.
Kaufman, J., Birmaher, B., Brent, D., Rao, U., Flynn, C., Moreci, P., ... Ryan, N. (1997). Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): Initial reliability and validity data. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(7), 980–988. https://doi.org/10.1097/00004583-199707000-00021.
Kovacs, E. (1984). The children's depression inventory (CDI). *Psychopharmacology Bulletin,* (21), 995–998.
Lansford, J. E., Criss, M. M., Dodge, K. A., Shaw, D. S., Pettit, G. S., & Bates, J. E. (2009). Trajectories of physical discipline: Early childhood antecedents and developmental outcomes. *Child Development, 80*(5), 1385–1402. https://doi.org/10.1111/j.1467-8624.2009.01340.x.
Lau, J. Y., Britton, J. C., Nelson, E. E., Angold, A., Ernst, M., Goldwin, M., ... Pine, D. S. (2011). Distinct neural signatures of threat learning in adolescents and adults. *Proceedings of the National Academy of Sciences of the United States of America, 108*(11), 4500–4505. https://doi.org/10.1073/pnas.1005494108.
Lau, J. Y., Lissek, S., Nelson, E. E., Lee, Y., Roberson-Nay, R., Poeth, K., ... Pine, D. S. (2008). Fear conditioning in adolescents with anxiety disorders: Results from a novel experimental paradigm. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*(1), 94–102. https://doi.org/10.1097/chi.0b01e31815a5f01 S0890-8567(09)62089-X [pii].
LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155–184. https://doi.org/10.1146/annurev.neuro.23.1.155.
LeDoux, J. E. (2014). Coming to terms with fear. *Proceedings of the National Academy of Sciences, 111*(8), 2871–2878.
Linnman, C., Rougemont-Bücking, A., Beucke, J. C., Zeffiro, T. A., & Milad, M. R. (2011). Unconditioned responses and functional fear networks in human classical conditioning. *Behavioural Brain Research, 221*(1), 237–245.
Lissek, S. (2012). Toward an account of clinical anxiety predicated on basic, neurally mapped mechanisms of Pavlovian fear-learning: The case for conditioned overgeneralization. *Depression and Anxiety, 29*(4), 257–263. https://doi.org/10.1002/da.21922.
Lissek, S., Powers, A. S., McClure, E. B., Phelps, E. A., Woldehawariat, G., Grillon, C., ... Pine, D. S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. *Behaviour Research and Therapy, 43*(11), 1391–1424. https://doi.org/10.1016/j.brat.2004.10.007.
Mackintosh, N. (1974). *The psychology of animal learning.* London: Academic Press.
MacMillan, H. L., Boyle, M. H., Wong, M. Y., Duku, E. K., Fleming, J. E., & Walsh, C. A. (1999). Slapping and spanking in childhood and its association with lifetime prevalence of psychiatric disorders in a general population sample. *CMAJ, 161*(7), 805–809.
Maheu, F. S., Dozier, M., Guyer, A. E., Mandell, D., Peloso, E., Poeth, K., ... Ernst, M. (2010). A preliminary study of medial temporal lobe function in youths with a history

of caregiver deprivation and emotional neglect. *Cognitive, Affective & Behavioral Neuroscience, 10*(1), 34–49. https://doi.org/10.3758/CABN.10.1.34.
Marek, R., Strobel, C., Bredy, T. W., & Sah, P. (2013). The amygdala and medial prefrontal cortex: Partners in the fear circuit. *Journal de Physiologie, 591*(Pt 10), 2381–2391. https://doi.org/10.1113/jphysiol.2012.248575.
McCrory, E. J., & Viding, E. (2015). The theory of latent vulnerability: Reconceptualizing the link between childhood maltreatment and psychiatric disorder. *Development and Psychopathology, 27*(2), 493–505. https://doi.org/10.1017/S0954579415000115.
McCrory, E. J., Gerin, M. I., & Viding, E. (2017). Annual Research Review: Childhood maltreatment, latent vulnerability and the shift to preventative psychiatry – The contribution of functional brain imaging. *Journal of Child Psychology and Psychiatry, 58*(4), 338–357. https://doi.org/10.1111/jcpp.12713.
McGuire, J. F., Orr, S. P., Essoe, J. K. Y., McCracken, J. T., Storch, E. A., & Piacentini, J. C. (2016). Extinction learning in childhood anxiety disorders, obsessive compulsive disorder and post-traumatic stress disorder: Implications for treatment. *Expert Review of Neurotherapeutics, 16*(10), 1155–1174. https://doi.org/10.1080/14737175.2016.1199276.
McLeod, B. D., Wood, J. J., & Weisz, J. R. (2007). Examining the association between parenting and childhood anxiety: A meta-analysis. *Clinical Psychology Review, 27*(2), 155–172. https://doi.org/10.1016/j.cpr.2006.09.002.
Milad, M. R., & Quirk, G. J. (2012). Fear extinction as a model for translational neuroscience: Ten years of progress. *Annual Review of Psychology, 63*, 129–151. https://doi.org/10.1146/annurev.psych.121208.131631.
Milad, M. R., Goldstein, J. M., Orr, S. P., Wedig, M. M., Klibanski, A., Pitman, R. K., ... Rauch, S. L. (2006). Fear conditioning and extinction: Influence of sex and menstrual cycle in healthy humans. *Behavioral Neuroscience, 120*(6), 1196.
Moran, G., Pederson, D., & Bento, S. (2009). *Maternal behavior Q-Sort (MBQS)–Overview.* Available Materials and Support.
Nagin, D. S. (2005). *Group-based modeling of development over the life course.* Cambridge.
O'Reilly, J. X., Woolrich, M. W., Behrens, T. E., Smith, S. M., & Johansen-Berg, H. (2012). Tools of the trade: Psychophysiological interactions and functional connectivity. *Social Cognitive and Affective Neuroscience, 7*(5), 604–609.
Pejic, T., Hermann, A., Vaitl, D., & Stark, R. (2013). Social anxiety modulates amygdala activation during social conditioning. *Social Cognitive and Affective Neuroscience, 8*(3), 267–276. https://doi.org/10.1093/scan/nsr095.
Perlman, G., Simmons, A. N., Wu, J., Hahn, K. S., Tapert, S. F., Max, J. E., ... Campbell-Sills, L. (2012). Amygdala response and functional connectivity during emotion regulation: A study of 14 depressed adolescents. *Journal of Affective Disorders, 139*(1), 75–84.
Petrovic, P., Carlsson, K., Petersson, K. M., Hansson, P., & Ingvar, M. (2004). Context-dependent deactivation of the amygdala during pain. *Journal of Cognitive Neuroscience, 16*(7), 1289–1301.
Pierce, T., Boivin, M., Frenette, E., Forget-Dubois, N., Dionne, G., & Tremblay, R. E. (2010). Maternal self-efficacy and hostile-reactive parenting from infancy to toddlerhood. *Infant Behavior & Development, 33*(2), 149–158. https://doi.org/10.1016/j.infbeh.2009.12.005 S0163-6383(09)00113-1 [pii].
Puetz, V. B., Viding, E., Palmer, A., Kelly, P. A., Lickley, R., Koutoufa, I., ... McCrory, E. J. (2016). Altered neural response to rejection-related words in children exposed to maltreatment. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 57*(10), 1165–1173. https://doi.org/10.1111/jcpp.12595.
Redlich, R., Stacey, D., Opel, N., Grotegerd, D., Dohm, K., Kugel, H., ... Dannlowski, U. (2015). Evidence of an IFN-γ by early life stress interaction in the regulation of amygdala reactivity to emotional stimuli. *Psychoneuroendocrinology, 62*, 166–173.
Silvers, J. A., Lumian, D. S., Gabard-Durnam, L., Gee, D. G., Goff, B., Fareri, D. S., ... Tottenham, N. (2016). Previous institutionalization is followed by broader amygdala–Hippocampal–PFC network connectivity during aversive learning in human development. *Journal of Neuroscience, 36*(24), 6420–6430. https://doi.org/10.1523/JNEUROSCI.0038-16.2016.
Simmons, W. K., Avery, J. A., Barcalow, J. C., Bodurka, J., Drevets, W. C., & Bellgowan, P. (2013). Keeping the body in mind: Insula functional organization and functional connectivity integrate interoceptive, exteroceptive, and emotional awareness. *Human Brain Mapping, 34*(11), 2944–2958.
Statistics Canada. 1995. Overview of the Canadian National Longitudinal Study ofChildren and Youth (NLSCY) survey instruments for 1994-95 data collection, cycle 1. Ottawa: Author.
Tanner, J. M. (1962). *Growth of adolescents.* Oxford: Blackwell Scientific Publications.
Tarabulsy, G. M. (2012). *Personal communication at the biannual conference of the International Society for the Study of Behavioral Development [ISSBD] in Edmonton Canada*.
Thomason, M. E., Marusak, H. A., Tocco, M. A., Vila, A. M., McGarragle, O., & Rosenberg, D. R. (2015). Altered amygdala connectivity in urban youth exposed to trauma. *Social Cognitive and Affective Neuroscience* nsv030.
Tovote, P., Fadok, J. P., & Luthi, A. (2015). Neuronal circuits for fear and anxiety. *Nature Reviews Neuroscience, 16*(6), 317–331. https://doi.org/10.1038/nrn3945.
Tzschoppe, J., Nees, F., Banaschewski, T., Barker, G. J., Buchel, C., Conrod, P. J., ... consortium, I. (2014). Aversive learning in adolescents: Modulation by amygdala-prefrontal and amygdala-hippocampal connectivity and neuroticism. *Neuropsychopharmacology, 39*(4), 875–884. https://doi.org/10.1038/npp.2013.287.
Van der Werff, S., Pannekoek, J., Veer, I., van Tol, M.-J., Aleman, A., Veltman, D., ... Van der Wee, N. (2013). Resting-state functional connectivity in adults with childhood emotional maltreatment. *Psychological Medicine, 43*(09), 1825–1836.
Varni, J. W., Thissen, D., Stucky, B. D., Liu, Y., Magnus, B., He, J., ... Amtmann, D. (2015). Item-level informant discrepancies between children and their parents on the PROMIS® pediatric scales. *Quality of Life Research, 24*(8), 1921–1937.
Wilke, M. (2012). An alternative approach towards assessing and accounting for individual motion in fMRI timeseries. *Neuroimage, 59*(3), 2062–2072. https://doi.org/10.1016/j.neuroimage.2011.10.043.
Wilke, M. (2014). Isolated assessment of translation or rotation severely underestimates the effects of subject motion in fMRI data. *PloS One, 9*(10), e106498. https://doi.org/10.1371/journal.pone.0106498.
Windham, A. M., Rosenberg, L., Fuddy, L., McFarlane, E., Sia, C., & Duggan, A. K. (2004). Risk of mother-reported child abuse in the first 3 years of life. *Child Abuse & Neglect, 28*(6), 645–667. https://doi.org/10.1016/j.chiabu.2004.01.003 S014521340400105X [pii].