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Physiological correlates of emotional reactivity and regulation in early adolescents

Melissa D. Latham[a], Nina Cook[b], Julian G. Simmons[b,c,d], Michelle L. Byrne[a], Jonathan W.L. Kettle[b], Orli Schwartz[b], Nandita Vijayakumar[a], Sarah Whittle[d], Nicholas B. Allen[a,b,c,*]

[a] Department of Psychology, University of Oregon, USA
[b] Melbourne School of Psychological Sciences, University of Melbourne, Australia
[c] Orygen Youth Health Research Centre, Centre for Youth Mental Health, University of Melbourne, Australia
[d] Melbourne Neuropsychiatry Centre, Department of Psychiatry, University of Melbourne, Australia

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ABSTRACT

Few studies have examined physiological correlates of emotional reactivity and regulation in adolescents, despite the occurrence in this group of significant developmental changes in emotional functioning. The current study employed multiple physiological measures (i.e., startle-elicited eyeblink and ERP, skin conductance, facial EMG) to assess the emotional reactivity and regulation of 113 early adolescents in response to valenced images. Reactivity was measured while participants viewed images, and regulation was measured when they were asked to discontinue or maintain their emotional reactions to the images. Adolescent participants did not exhibit fear-potentiated startle blink. However, they did display affect-consistent zygomatic and corrugator activity during reactivity, as well as inhibition of some of these facial patterns during regulation. Skin conductance demonstrated arousal dependent activity during reactivity, and overall decreases during regulation. These findings suggest that early adolescents display reactivity to valenced pictures, but not to startle probes. Psychophysiological patterns during emotion regulation indicate additional effort and/or attention during the regulation process.

1. Introduction

Reactions to emotional stimuli, and the ability to regulate those reactions, are important to human functioning and are strong determinants of adaptive behavior across a range of domains, including mental health (Gross & Jazaieri, 2014), physical health (Isasi, Ostrovsky, & Wills, 2013), and school functioning (Schelble, Franks, & Miller, 2010). A common and important form of emotion regulation relates to the ability to effortfully change the expression and/or intensity associated with specific emotions (Gross & Jazaieri, 2014). Early adolescence is an especially important time to study this phenomenon, since it is a critical period for change in emotional reactivity and the emergence of affect regulation skills, especially related to increased ability to self-regulate (Riediger & Klipker, 2014), as well as a period of high risk for the onset of disorders of emotion (Allen & Sheeber, 2008). This suggests that understanding the processes associated with emotional reactivity and regulation in this age group will likely have important implications for affective, developmental, and clinical science.

The psychobiological processes that underlie emotional reactivity and regulation in healthy adolescents are still not fully understood, despite much literature asserting the importance of these processes for emotional development during this phase of life (McLaughlin, Hatzenbuehler, Mennin, & Nolen-Hoeksema, 2011). However, there is some research addressing how this developmental stage differs from others. Compared to children, adolescents are better able to identify their emotions, deal proactively with emotional responses, and adapt emotion regulation strategies to diverse situations (Riediger & Klipker, 2014). Research on emotion regulation strategies suggests that adolescents use more proactive regulation strategies as they age, such as planful problem solving. An analysis of 58 studies on emotion regulation across the lifespan showed that adolescents are able to use both behavioral and cognitive regulation strategies (Zimmer-Gembeck & Skinner, 2011). There was also evidence from these studies that, although adolescence as a whole is a period of emotion regulation development, early adolescents may regress, demonstrating less

* Corresponding author at: Department of Psychology, University of Oregon, Eugene, OR 97403-1227, USA.
E-mail address: nallen3@uoregon.edu (N.B. Allen).

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effective emotion regulation and more intense reactivity to stress than in late childhood or late adolescence (Zimmer-Gembeck & Skinner, 2011).

Much of what we know about emotion regulation in adolescence comes from the research on cognitive control and self-regulation (Riediger & Klipker, 2014). Some literature suggests that the frontal brain regions associated with cognitive control are also activated during emotion regulation tasks (e.g., Mauss, Bunge, & Gross, 2007; Ochsner & Gross, 2005). One often-cited theory is that the imbalanced development of limbic and frontal brain areas during adolescence results in the emotional difficulties often observed during this developmental period. Once frontal brain areas catch up to the more 'emotional' limbic areas such as the amygdala, adolescents are better able to regulate their emotional responses (Casey, Jones, & Hare, 2008). It has also been theorized that the development of these frontal regions, and thus the ability to forecast and plan for the future, also contributes to difficulties in emotion regulation, partially due to the vast amount of new situations to which adolescents can now attend and form reactions (Pfeifer & Allen, 2012).

Despite the array of data on emotion reactivity and regulation in adolescents, no study to our knowledge has investigated these processes using psychophysiological measurements and paradigms. These include affective picture viewing paradigms that examine startle eyeblink modulation, facial muscle activity, skin conductance, and neurophysiological responses. These methods of assessment each yield different and complementary information, resulting in a richer understanding of emotional responses, as is detailed below. The addition of this information to the current knowledge on these processes in adolescence is an important next step in understanding emotional processes for this vulnerable age group. We will describe the common findings for each of these methods of assessment, and highlight studies that have utilized these methods with picture-viewing paradigms. This will be detailed first for emotional reactivity and later for emotional regulation.

1.1. Emotional reactivity

Startle eyeblink and facial muscle activity are both hypothesized to provide differing information dependent on the valence of the emotional stimuli being presented. The majority of findings from startle blink paradigms while viewing emotional stimuli corroborate the concept of *fear-potentiated startle*, which dictates that the startle blink is of the largest magnitude when viewing unpleasant stimuli (Bernat, Cadwallader, Seo, Vizueta, & Patrick, 2011; McManis, Bradley, Berg, Cuthbert, & Lang, 2001). Fear-potentiated startle represents a learned, automatic response to fear-invoking stimuli, a learning process that is at least partially dependent on amygdala functioning (Klumpers, Morgan, Terburg, Stein, & van Honk, 2015). This finding is also present in more traditional fear paradigms, such as using threat or darkness (Balaban & Berg, 2008). Startle blink magnitude is commonly attenuated when viewing neutral and pleasant pictures (Bernat et al., 2011).

Affective facial muscle activity is commonly operationalized by measuring the *corrugator supercilii* ('frown') and *zygomaticus major* ('smiling') muscles. Research examining reactivity to valenced pictures typically yields a pattern one would expect – 'frown' muscles are more commonly activated when viewing unpleasant stimuli, while 'smile' muscles are activated when viewing stimuli that are pleasant (Bernat et al., 2011; Lang, Greenwald, Bradley, & Hamm, 1993).

Skin conductance and neurophysiological responses provide information on arousal levels of stimuli irrespective of their affective valence. In general, greater levels of skin conductance indicate higher levels of arousal, and are common when participants view either highly pleasant *or* unpleasant images, while neutral pictures elicit lower levels (Bernat et al., 2011; Gross, 1998). Neurophysiological responses measured via startle probe-elicited cortical event related potentials (ERPs) also commonly demonstrate lesser amplitude of the P300 ERP component when viewing pleasant *or* unpleasant stimuli as compared to neutral stimuli (Bernat et al., 2011; Cuthbert, Schupp, Bradley, McManis, & Lang, 1998). The P300 component is believed to reflect the amount of attention paid to the startle stimulus (in this case, an auditory startle probe) when it is displayed secondary to an affective foreground stimulus (the goal-relevant stimulus; in this case, affective pictures). The implication is that paying more attention to the startle probe indicates that less attention is being paid to the competing cross-modal foreground stimulus.

These methods allow the interpretation of stimulus processing as it varies by valence and arousal. However, the majority of this information has come from adult studies. Research on the physiology of emotional reactivity in children gives a picture of how these methods might yield differing results in younger, still developing groups. In the only study we are aware of that has used multiple methods to assess affective reactivity in children, McManis et al. (2001) found greater skin conductance for seven- to ten-year-old girls than boys, especially when viewing unpleasant stimuli, and an increase in corrugator ('frown' muscle) activity for both genders when viewing unpleasant stimuli (with a greater increase for girls; McManis et al., 2001). Another common finding in this literature is that children do not display fear-potentiated startle in response to aversive stimuli (McManis et al., 2001; Van Brakel, Muris, & Derks, 2006), and a study on adolescents (mean age 16) also failed to observe this effect (Nederhof, Creemers, Huizink, Ormel, & Oldehinkel, 2011). However, there are notable exceptions to that pattern.

Despite a number of studies failing to observe fear-potentiated startle in younger samples, it should be noted that this effect has been demonstrated in certain studies (Quevedo, Smith, Donzella, Schunk, & Gunnar, 2010; Schmitz, Grillon, Avenevoli, Cui, & Merikangas, 2014). Of course there are a number of methodological differences between studies of affective startle modulation in younger samples that might explain these different findings. For example, studies have varied in terms of the age of participants (e.g., 3–9 in Quevedo et al., 2010 versus 16 in Nederhof et al., 2011) and the intensity of the startle probe (e.g., 95 dB in Van Brakel et al., 2006; McManis et al., 2001, and 105 dB in Waters, Lipp, & Spence, 2005).[1] However, these methodological variations do not appear to explain the presence or absence of fear-potentiated startle across these studies. The studies also differ in their specific stimulus paradigms. In particular, studies that employ picture viewing methods and present startle probes while viewing these images (whether affectively charged scenes or faces), tend not to find fear-potentiated startle in younger participants (McManis et al., 2001; Nederhof et al., 2011; Van Brakel et al., 2006). On the other hand, those studies that have observed fear-potentiated startle in youth used non-picture stimuli such as movie clips (Quevedo et al., 2010) or air blasts as the affective stimuli (Schmitz et al., 2014). Thus, it seems that paradigms that include more intense, threatening stimuli are more likely to yield findings of fear-potentiation in younger samples, despite the broader range of threatening stimuli that evokes this response in adults.

1.2. Emotional regulation

The measures described above have also been used to assess physiological markers of emotion regulation. In these studies participants are instructed to enhance or suppress their emotional reactions to stimuli, but the methods by which they do so are often self-determined. Thus, these studies do not directly assess cognitive emotion regulation strategies, such as those described by Gross (Gross, 1998) but rather examine the behavioral and physiological correlates of explicit efforts

[1] The study by Waters et al. (2005) did observe fear-potentiated startle for startle probes presented 60 ms following picture onset. Longer lead times that were similar to those examined in the other studies did not show evidence of affective startle modulation.

to alter emotional experience and expression, regardless of the strategy used. For this reason, participants are often asked to merely increase or decrease the strength of their emotional feeling (Baur, Conzelmann, Wieser, & Pauli, 2015; Bernat et al., 2011). Startle eyeblink reflexes are consistently largest when one is voluntarily enhancing emotional responses to affective stimuli and smallest when voluntarily suppressing these responses (Bernat et al., 2011; Dillon & Labar, 2005). When instructed to increase or decrease emotions elicited by affective pictures, facial muscle activity fluctuates in a predictable way, with greater corrugator activity for increase of negative emotions and greater zygomatic activity increase of positive emotions. Decrease for each emotion shows the opposite pattern (Baur et al., 2015). Skin conductance shows a general pattern of increase both under enhancement and suppression conditions (Bernat et al., 2011; Gross, 1998), possibly reflecting the effort required to regulate emotional states. Neurophysiological responses can provide insight into attentional focus during effortful emotion regulation. Studies have shown that the amplitude of the P300 component of the startle-elicited ERP is diminished during suppression and enhancement conditions, presumably because more attention is being paid to the internal processes associated with modulating the emotional state and, therefore, less to the startle probe (Bernat et al., 2011). As with the literature on emotional reactivity, these findings come primarily from studies with adult samples.

As with the literature on reactivity, the limited amount of knowledge we have about regulation processes in younger age groups comes from research on children. Findings suggest that one primary means of controlling emotions in this age group may be through the use of facial expressions. Ceschi and Scherer (2003) found that, when asked to suppress smiles, seven- and ten-year-olds were unable to completely suppress, but did smile for shorter periods of time than if they were not asked to suppress. The study reported that there was no difference in suppression strategies between seven- and ten-year-olds, but there was significantly more residual smiling during the suppression condition amongst the seven-year-olds, indicating that the ability to suppress facial expressions may improve with age (Ceschi & Scherer, 2003). Bar-Haim, Bar-Av, and Sadeh (2011) also found that six-year-olds are able to suppress their facial expressions when instructed to do so, during both successful and unsuccessful trials of a computer game. These findings are consistent with the notion that younger children tend to regulate their emotions by controlling expressive behavior, possibly more so than through the use of mentalistic strategies, such as attention shifting and reappraisal (John & Gross, 2004). Unfortunately, very few physiological studies have been conducted to examine emotion regulation in children and, to our knowledge, there is not currently data using multiple methods to assess adolescents' physiological responses while experiencing or regulating emotional reactions.

Our goal in measuring emotion regulation is to assess physiological correlates of natural and spontaneous forms of emotion regulation. For that reason, we employ a paradigm with simple regulation instructions based on the paradigm for adults used by Jackson, Malmstadt, Larson, and Davidson (2000). We asked participants in our study to "stop" or "continue" feeling the emotion that each picture elicited. The "continue" instruction is meant to facilitate maintenance of the emotion that participants are feeling, as well as its intensity. The "stop" instruction is meant to facilitate regulation of that emotion. In this case, our regulation task is specific to down-regulation and does not contain specific instructions on how to achieve that down-regulation, besides instructing participants to continue paying attention to the image (and thus not distracting themselves).

The aim of this study was to broadly describe the physiological correlates of affective reactivity and regulation in a group of early adolescents using four different methods of physiological assessment. Despite the wide-spread study of specific emotion regulation techniques, our objective was to measure physiology related to early adolescents' reactivity and their baseline methods for regulating their emotions. Given that they were not instructed to regulate in any particular way, we believe this design will yield ecologically valid measures. Based on the previous, albeit limited, findings in this area, it was hypothesized that early adolescents would show differential physiological responses to putatively pleasant, neutral, and unpleasant affective pictures during the reactivity phase. This includes facial muscle action (i.e., increased corrugator activity during unpleasant pictures, and increased zygomatic activity during pleasant pictures), heightened skin conductance, and inhibition of the startle-elicited P300 while viewing affective pictures (i.e., pleasant or unpleasant). We also hypothesized that we would not observe fear-potentiated startle given previous studies that failed to observe this effect in picture viewing studies with younger samples. Furthermore, it was hypothesized that early adolescents' emotion-related physiology would change as a result of their efforts to regulate affective responses; however given the absence of previous literature addressing these processes using psychophysiological measures within picture viewing paradigms in youth, we treated this as an exploratory aim to describe the specific physiological correlates of emotion regulation in this age group.

2. Method

2.1. Participants

Participants were recruited from the Orygen Adolescent Development Study, a longitudinal, multi-method study in Melbourne, Australia. Further details about recruitment and selection of participants in this study can be found elsewhere (Whittle et al., 2008). 2453 participants were screened at age 11 using the Early Adolescent Temperament Questionnaire – Revised. 414 participants were then selected to participate in a longitudinal study based on oversampling participants at the extreme ends (high and low) of Negative Emotionality and Effortful Control, in order to enhance phenotypic risk for the incidence of future psychopathology, consistent with the prospective aim of the larger study. Of 240 eligible participants who agreed to participate in the longitudinal study, 113 (58 males, mean age = 12.62 years, SD = .48; 56 females, mean age = 12.74 years, SD = .38) were randomly allocated and consented to complete this psychophysiological assessment at the first time point.

2.2. Materials

2.2.1. Affective picture set

The picture set contained 18 unpleasant, 18 pleasant, and 18 neutral pictures. The unpleasant and pleasant pictures were selected on the basis of valence and arousal ratings collected in a previous pilot study, and neutral pictures were selected in part from pilot pictures that were rated low on arousal and neutral in valence and in part from previous picture viewing studies with adults and children (McManis et al., 2001). Valence ratings were significantly more negative for unpleasant (M = 3.26, SD = .50) than for pleasant (M = 6.96, SD = .78) pictures, $t(19) = 17.17$, $p < .001$, with no significant difference between the unpleasant (M = 6.41, SD = .87) and pleasant (M = 6.64, SD = .73) picture categories for arousal ratings ($t(19) < 1$), and no interactions with gender for either valence or arousal ratings ($F(1,18) = .03$ and 1.17 respectively, both n.s.). Each picture was presented to participants for eight seconds on a 21-inch CRT Sony Trinitron monitor, placed approximately 1 m from participants' knees, such that pictures comprised approximately 24° of visual angle.

2.2.2. Emotion regulation instructions and startle probes

Instructions to 'stop' and 'continue' emotional responses were recorded to be as acoustically consistent as possible, and were presented binaurally through Sennheiser HD 280 Pro headphones, at 4 s after picture onset. A binaural, acoustic startle probe, consisting of a 50msec burst of 95 dB white noise with immediate rise time, was presented either before the instruction (i.e., at 2.5 s after picture onset – 'probe

one,' present on one-third of trials), after the instruction (i.e., at 6.5 s after picture onset – 'probe two,' present on one-third of trials), or in both of these positions (the remaining trials). Startle probes were presented at either 5 or 7 s post picture offset for 18 trials as an inter-trial interval measure. The picture and startle probe orders were counterbalanced across participants.

2.3. Procedure

Participants attended the session with at least one parent or guardian. The experimenter described the procedure, potential risks and benefits, confidentiality, and the voluntary nature of the research, and both the parent/guardian and child gave informed consent.

Skin conductance electrodes were applied to the non-dominant hand, facial electrodes applied to the participant's face, and an EEG cap applied and electrodes filled with conducting gel. Inter electrode impedance were kept below 10 kilohms wherever possible, although some signals where impedances were slightly greater than this were retained for analysis if the signals were clear and free of noise. Participants were taken into a separate, sound attenuated room and completed the Positive and Negative Affect Schedule (PANAS), a measurement of overall affect (Watson, Clark, & Tellegen, 1988). Participants were instructed to indicate how often they were feeling different positive and negative emotions, from "very slightly" to "very much," on a 1–5 Likert scale. This measure has been demonstrated to be reliable in children (Laurent, Catanzaro, & Joiner, 2004).

Participants were then instructed to attend to each of the pictures and their emotional reaction to it, and to endeavour to 'stop' or 'continue' that emotional reaction as instructed. Participants were coached in the emotion regulation task using a personally relevant example. Instruction included the following script: "It is important that you watch each picture the whole time it is on the screen, without closing your eyes or looking away… If the emotion you experience in response to a picture is happiness and you are instructed to STOP, we would like you to feel less happy… If the emotion you are feeling in response to a picture is happiness, and you are instructed to CONTINUE this emotion, we would like you to keep feeling the same amount of happiness… This can be pretty tricky, but we want to see how well you can follow the instructions, so try your best even if it's hard." To illustrate the task, the experimenter described a personally meaningful example of a pleasant picture, e.g. asking the participant to imagine how they would feel if they saw a picture of their favorite football team winning a big game, and then asked what they would do if asked to 'stop' or 'continue' that feeling. The participant was coached on their understanding of the emotion regulation task as required, but the experimenter did not suggest specific emotion regulation strategies. Two sample pictures, one pleasant with a 'stop' instruction and one unpleasant with a 'continue' instruction, were presented. The experimenter checked with the participant whether he or she was able to follow the instructions. If the experimenter was concerned that the participant did not fully understand the task, another two sample pictures were presented, and further coaching was provided. If the experimenter felt that the participant fully understood the task after the first two sample pictures, the remaining two sample pictures were shown, but no further coaching was given prior to the start of the experimental procedure.

Participants were instructed to ignore the startle probes on all trials. At the conclusion of the picture-viewing program, participants were debriefed and reimbursed with a $30 (AUD) gift voucher and parents/guardians reimbursed $50 (AUD) cash.

2.4. Data processing and reduction

Physiological signals were recorded using a Grass Model 12 Neurodata acquisition system linked to an IBM compatible microcomputer via a PC-Labcard 812-PG analog-to-digital converter. The VPM 11.0 software package (Cook, 2000) controlled the timing and presentation of stimuli, and collection and storage of the physiological data. Facial EMG activity was recorded over the left *zygomaticus major* and *corrugator supercilii* muscles (Tassinary, Cacioppo, & Green, 1989). Facial EMG signals were amplified by a factor of 100,000 and half amplification high and low pass filters set to 30 Hz and 1 kHz, respectively. Facial EMG signals were sampled at 1000 Hz from 2 s prior to picture onset through to the end of the 8-s picture presentation period. In order to smooth the signal to quantify overall activity at each facial muscle site, the signals were integrated separately (full-wave rectified), converted to microvolts, and filtered using a digital Finite Impulse Response (FIR), 50 Hz, 24/dB rolloff Zero Phase Shift low pass filter in Neuroscan v. 4.3. EMG data points were then smoothed across 20 ms time points and corrected to the mean of a 1 s, pre-picture onset baseline via calculation of a difference score. Mean EMG activity was then computed for two time periods: 1–4 s (pre-instruction or reactivity) and 5–8 s (post- instruction or regulation) post picture onset. Facial EMG activity was averaged for each time period within participants, within each picture type for emotional reactivity and within picture type by instructions category for emotional regulation.

Skin conductance activity was recorded via bipolar Ag/AgCl electrodes, placed on the volar surfaces of the medial phalanges of the first and second fingers of the non-dominant hand, using Velcro finger straps. Signals were recorded using a Grass Model SCA1 Skin Conductance Adaptor interfaced to a Grass Polygraph DC Driver Amplifier. The voltage provided across electrodes was adjusted according to the strength of the signal (sensitivity). Skin conductance response was scored as the largest (peak) value in μSiemens for the pre-instruction period (picture onset to 4 s) or the post-instruction period (4–8 s post picture onset), minus the mean response for the 4 s pre-picture period, and adjusted for sensitivity. Skin conductance scores were then averaged for each participant and picture category (unpleasant, neutral, pleasant).

Magnitude of the startle eyeblink component was measured by recording the EMG activity of the *orbicularis oculi* muscle beneath the left eye. A pair of 6 mm Ag/AgCl miniature electrodes was placed approximately 0.8 cm under the pupil and on the outer canthus of the eye. The raw EMG signal was amplified by 50,000 and half amplification high and low pass filters set to 30 Hz and 1 kHz, respectively. Blink magnitudes were sampled at 1000 Hz from 50 ms prior to until 250 ms after startle probe onset. Digitized raw EMG signals were integrated (full-wave rectified) and filtered offline with a 50 Hz, low pass filter. These integrated blink responses were scored for startle magnitude, onset and peak latency with VPMANLOG (Cook, 2000), using the algorithm of Balaban and colleagues (Balaban, Losito, Simons, & Graham, 1986), and converted to microvolts. Algorithm parameters were set such that the program scanned for the first startle onset between 20 and 120 ms after probe onset and identified a peak startle response within 150 ms, in accordance with startle reflex publication guidelines (Blumenthal et al., 2005). The maximum time from response onset to peak was 95 ms. The algorithm scored up to two responses per trial, and each trial was visually inspected and adjusted when appropriate. Startle magnitude was computed within conditions of picture type (probe 1) and picture type and instructions (probe 2).

The electroencephalogram (EEG) was recorded from 9 scalp sites, based on the international 10–20 system – frontal (F3, Fz, F4), central (C3, Cz, C4), and parietal (P3, Pz, P4), with linked earlobes as the reference and forehead as the ground. Vertical electrooculography (EOG) was recorded from electrodes placed above and below the right eye, and horizontal EOG from electrodes placed at the outer canthi of each eye. The raw EEG and EOG signals were amplified by 10,000 and half amplification high and low pass filters set to .1 Hz and 30 Hz, respectively. Data were collected at 1000 Hz from 2 s before picture onset (baseline period) until 2.5 safter the second probe (i.e., 9 s after picture onset) for all 54 trials. EEG data chunks were extracted for 1 s prior, to 1.25 s post, probe onset, converted to microvolts, and analysed using Neuroscan v.4.3. For each probe presentation, EEG and EOG recordings

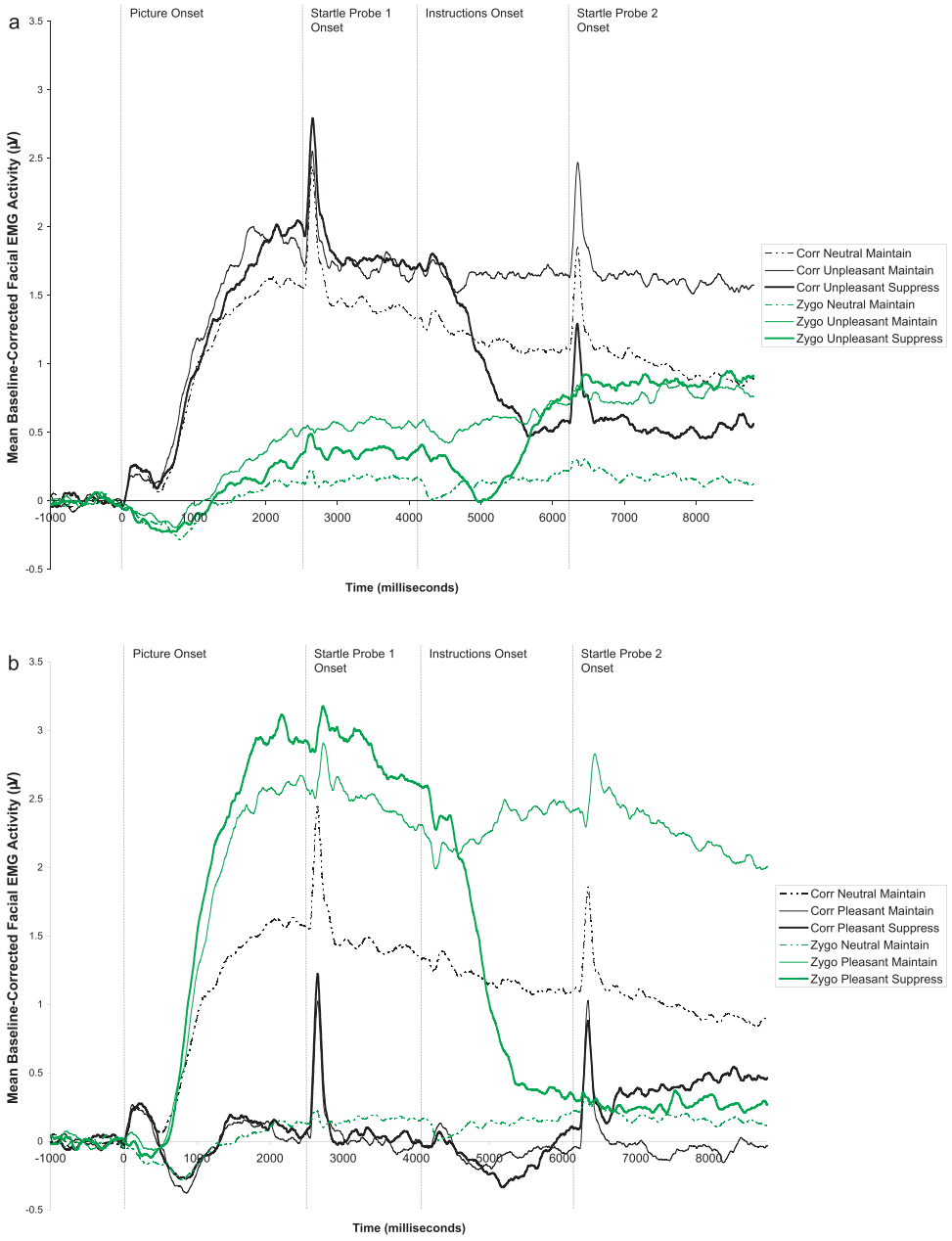

Fig. 1. Continuous corrugator and zygomatic EMG activity during emotional reactivity and regulation for (a) unpleasant and neutral pictures (top panel); and (b) pleasant and neutral pictures (bottom panel).

were manually examined for saturation (greater than +75 μV), with saturated trials excluded from further analyses. All channels were then baseline corrected to the mean of their 150 ms pre-stimulus period. An eye movement artefact correction algorithm (Semlitsch, Anderer, Schuster, & Presslich, 1986) using VEOG channel data was applied within participants for each trial for each EEG channel to correct for vertical ocular artefacts. All data trials were then re-examined and any further saturated trials or outliers were excluded. Event-related potential waveforms were then obtained for each individual by averaging the EEG signal at each scalp site for each picture category (and for each emotion regulation instruction for probe two). Startle-elicited N1, P2, N2, and P3 components were identified by combining visual inspection of grand averaged waveforms and a peak-scoring algorithm written in our laboratory, which used the component parameters of Schupp and colleagues (1997). These components fall in the following time windows: N1 (64–192 ms), P2 (from N1 latency until 272 ms), N2 (from P2 latency until 336 ms) and P3 (from N2 latency until 504 ms). Only the startle-elicited P300 component was analysed, given that this is the most researched and functionally well-understood probe-elicited ERP component (e.g., Schupp, Cuthbert, Bradley, Birbaumer, & Lang, 1997).

2.5. Data analysis

Statistical analysis was undertaken in SPSS. The multivariate statistic Wilks' lambda was used for all tests to protect against violations of sphericity. Statistical significance was set at $p < 0.05$. Normality of variables was assessed using visual inspection of histograms and indices of skewness ($< 3.29 * \text{sqrt}[6/n]$). Univariate outliers were cases more than 3.29 standard deviations greater or lesser than the mean, and multivariate outliers were assessed using a critical Mahalanobis distance value of χ^2 (df = number of independent variables), $p = .001$ (Tabachnick & Fidell, 1996). Non-normally distributed variables were transformed and outliers brought in to be .01 unit greater or lesser than the extreme ends of the distribution. Homogeneity of variance assumptions were assessed using Levene's Test of Equality of Error Variances, for each repeated-measures condition of the variable, with a statistical significant level of $p < .001$.

Participants were excluded from corrugator analyses ($n = 7$),

zygomatic analyses (n = 7), and SCR analyses (n = 8) due to equipment malfunction. Participants were excluded from startle analyses due to equipment malfunction (n = 7) or if they exhibited a scorable startle response on less than 25% of trials (startle inclusion criterion; n = 56; reasons for this high rate of startle exclusion included noisy signals and unstable baselines associated with movement artefact, which may be more common among young samples – see discussion). For probe 1 elicited ERPs, participants were excluded due to equipment malfunction (n = 1) or if they had less than five valid trials for each picture type (n = 3). For probe two elicited ERPs, participants were excluded due to equipment malfunction (n = 1) or if they had less than two valid trials in any picture valence by instructions condition (n = 6). The average percent of trials excluded from ERP analyses was 34.45% for Probe 1 and 19.86% for Probe 2. Average waveforms were based on an average of 7.9 trials for Probe 1 and, for Probe 2, 5.1 trials for unpleasant/pleasant and maintain/suppress conditions and 8.5 trials for the neutral maintain condition. Startle-elicited P300 amplitude was analysed at parietal sites, given past research indicating effects are strongest at parietal sites.

Data analyses focused on basic effects of emotional reactivity and regulation. Emotional reactivity was analysed within the first four seconds of picture viewing and emotional regulation within the last four seconds of picture viewing, after the onset of instructions. Facial EMG, SCR, and startle reflex, were all analysed using three-way, mixed factor ANOVAs (picture [3: unpleasant, neutral, pleasant] × gender [2: male, female] × picture order [2: Order1, Order 2]), and startle-elicited P300 was analysed using a two-way, mixed factor ANOVA (picture [3: unpleasant, neutral, pleasant] × gender [2: male, female]).

For emotional regulation, the facial EMG activity, SCR, and startle reflex, were analysed using four-way, mixed-factor ANOVAs (picture [2: unpleasant, pleasant] × instructions [2: maintain, suppress] × gender [2: male, female] × picture order [2: Order1, Order 2]). Startle-elicited P300 at Pz was analysed using three-way, mixed-factor ANOVAs (picture [2: unpleasant, pleasant] × instructions [2: maintain, suppress] × gender [2: male, female]). Planned, simple contrasts were performed to address significant main effects and interactions. Effect size was indexed by partial eta squared.

3. Results

3.1. Emotional reactivity

3.1.1. Corrugator reactivity

As described in Fig. 1, the main effect of picture was significant $F(2, 100) = 4.68$, $p = .011$, partial $\eta^2 = .086$, with corrugator activity significantly lower for pleasant compared to neutral and unpleasant pictures. There was no difference between neutral and unpleasant pictures, and no other significant main effects or interactions (see Supplementary Table S1).

As described in Fig. 1, the main effect of picture was significant $F(2, 99) = 4.402$, $p < .015$, partial $\eta^2 = .082$, with mean zygomatic activity significantly greater for pleasant compared to neutral and unpleasant pictures. No other main effects or interactions were significant (see Supplementary Table S2).

3.1.2. SCR reactivity

There was a significant main effect of picture, $F(2, 92) = 5.777$, $p = .004$, partial $\eta^2 = .112$. As shown in Fig. 2, SCR amplitude was significantly greater for unpleasant compared to neutral, $F(1, 93) = 11.667$, $p = .001$, partial $\eta^2 = .111$, and pleasant compared to neutral pictures, $F(1, 93) = 7.471$, $p = .008$, partial $\eta^2 = .074$, with no significant difference between unpleasant and pleasant pictures, $F < 1$. All other main effects and interactions were not significant.[2]

[2] Visual examination of Fig. 2 suggests a difference in SCR values for unpleasant picture conditions between maintain and suppress at 4 s, i.e., before the instruction was given. Upon further examination, there is a statistically significant difference between the SCR values for these two conditions immediately prior to the instructions, which cannot be explained by the experimental procedure, given that trials are identical before the instructions. We have examined whether some aspects of data processing (i.e., filtering) could have resulted in the post instruction effects influencing pre-instruction values, but have been unable to find evidence of such effects. Additionally, the effect is only true for unpleasant picture conditions, ruling out the possibility of valence causing this difference. As such we have concluded that the effect is a result of type one error.

3.1.3. Startle reflex reactivity

There was a significant main effect of picture, $F(2, 50) = 5.714$, $p < .01$, partial $\eta^2 = .186$. Startle reflex magnitude was significantly attenuated during unpleasant compared to neutral picture viewing, $F(1, 75) = 11.210$, $p = .002$, partial $\eta^2 = .180$, with no significant difference between pleasant and neutral pictures, $F < 1$. There was no significant main effect of gender or interaction between gender and picture type, $Fs < 1$. See Table 1 for means and standard deviations for all startle conditions.

3.1.4. Startle-elicited P300 reactivity at Pz

The main effect of picture was not significant, $F(2, 62) = 2.595$, $p = .083$, partial $\eta^2 = .077$. However, testing a priori hypotheses about P300 attenuation to affective pictures, startle-elicited P300 amplitude was significantly attenuated for pleasant compared to neutral pictures, $F(1, 63) = 5.214$, $p = .026$, partial $\eta^2 = .076$, but not for unpleasant compared to neutral pictures $F(1, 63) = 3.017$, $p = .087$, partial $\eta^2 = .046$, as shown in Fig. 3. The main effect of gender, $F < 1$, and interaction between picture and gender, $F(2, 62) = 1.451$, $p = .242$, partial $\eta^2 = .045$, were not significant.

3.2. Emotion regulation

Corrugator Regulation. The main effect of picture was significant $F(1, 101) = 4.873$, $p = .030$, partial $\eta^2 = .046$, with mean corrugator activity significantly attenuated for pleasant pictures compared to unpleasant pictures. However, this was qualified by significant picture by instruction $F(1, 101) = 4.030$, $p = .038$, partial $\eta^2 = .047$, and picture by gender $F(1, 101) = 3.699$, $p = .038$, partial $\eta^2 = .049$, interactions (see Supplementary Table S3). Inspection of estimated means revealed that the difference between corrugator activity while viewing pleasant and unpleasant pictures was greater during the maintain instruction than the suppress instruction, and greater amongst females than males. Differential effects of instructions according to picture type are displayed in Fig. 1.

3.2.1. Zygomatic regulation

The main effect of picture was significant $F(1, 101) = 4.453$, $p = .037$, partial $\eta^2 = .043$, with mean zygomatic activity significantly potentiated while viewing pleasant pictures compared to unpleasant pictures. There were no other significant main effects or interactions (see Supplementary Table S4).

3.2.2. SCR regulation

There was a significant main effect of instruction, $F(1, 93) = 11.191$, $p < .001$, partial $\eta^2 = .107$. As shown in Fig. 2, SCR amplitude was significantly attenuated following suppress compared to maintain instructions. All other main effects and interactions for SCR were not significant[2].

3.2.3. Startle reflex regulation

The main effect of picture was not significant, $F(1, 51) = 2.774$, $p = .10$, partial $\eta^2 = .052$, however the main effect of instructions was significant, $F(1, 51) = 21.771$, $p = .001$, partial $\eta^2 = .299$. Startle reflex magnitude was significantly attenuated following suppress compared to maintain instructions. All other main effects and interactions

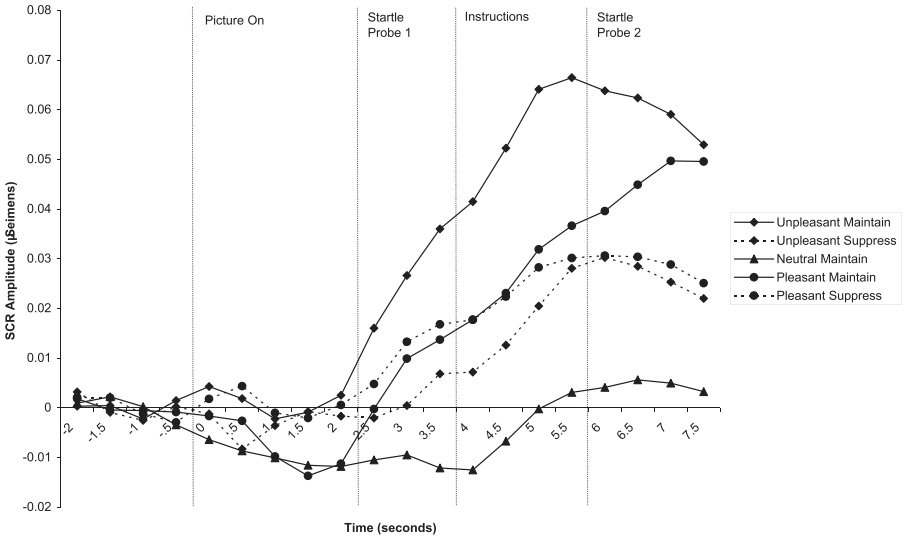

Fig. 2. Continuous SCR reactivity by picture type and emotion regulation instructions.

Table 1
Means (standard deviations) in microvolts for raw startle blink EMG reactivity by affective condition and gender.

Affective Stimuli	Males	Females	All
Pleasant	156.93 (184.10)	165.38 (223.74)	161.04 (203.45)
Neutral	151.31 (182.77)	172.55 (244.37)	161.65 (214.23)
Unpleasant	138.96 (178.48)	144.49 (201.03)	141.65 (188.93)

for startle magnitude were not significant. See Table 2 for means and standard deviations for all startle conditions.

3.2.4. Startle-elicited P300 ERP Regulation at Pz

There was a significant main effect of instruction, $F(1, 63) = 4.643$, $p = .035$, partial $\eta^2 = .069$, as shown in Fig. 3. Startle-elicited P300 amplitude was significantly attenuated following suppress compared to maintain instructions. All other main effects and interactions were not significant.

4. Discussion

The purpose of the present study was to provide a description of the physiological correlates of emotional reactivity and regulation in early adolescence. Although early adolescents do not show fear-potentiated startle, they do exhibit affect-modulated reactivity in other physiological measures, and changes to their physiology during effortful emotion regulation indicate increased effort and/or attention to the regulation process.

As hypothesized, participants did not display fear-potentiated startle, but rather showed attenuated startle blink while viewing unpleasant pictures compared to pleasant and neutral pictures, especially earlier in picture view (prior to the maintain/suppress instruction). In addition, the amplitude of the startle-elicited P300 was only inhibited while viewing pleasant pictures compared to neutral and unpleasant pictures. However, both facial muscle actions and skin conductance demonstrated patterns that were valence and arousal dependent, respectively. Data on emotion regulation indicated lower startle-blink amplitude and attenuated startle-elicited ERP during the “stop” condition compared to the “continue” condition, as well as deactivation of relevant facial muscles, especially corrugator. Decreased skin conductance was found in adolescents during emotion regulation irrespective of picture viewing condition.

4.1. Emotional reactivity

The pattern of findings for early adolescents’ emotional reactivity sheds light on their physiological responses to valenced and neutral stimuli. Corrugator activity was greater during unpleasant pictures than neutral or pleasant pictures. Zygomatic activity showed the opposite pattern, with overall greater activity during pleasant pictures. Skin conductance also showed the hypothesized pattern, with greater conductance in response to both positive and negative valenced pictures compared to neutral pictures. Previous studies suggest that this demonstrates an arousal response to both positive and negative affective stimuli, which is consistent with the relationship between skin conductance and the sympathetic nervous system (Bernat et al., 2011; Gross, 1998). These findings are what we would expect based on previous literature, but provide new insight into affective reactivity in adolescents. It seems that adolescents respond to pleasant, unpleasant, and neutral images in ways that are consistent with those images’ valence and arousal.

The two measures that specifically assessed responses to the startle probe showed a pattern of response that corresponds to some previous research with children. Studies that have implemented picture-viewing paradigms with younger samples have often failed to detect fear-potentiated startle (McManis et al., 2001; Nederhof et al., 2011; Van Brakel et al., 2006). This finding was also true in the present sample of early adolescents, as the startle-elicited blink was attenuated while viewing unpleasant pictures compared with neutral and pleasant pictures, especially during early picture processing.

In addition, early adolescents did not display a pattern of inhibition of the startle-elicited ERP at P300 while viewing unpleasant pictures, although they did display inhibition while viewing pleasant pictures. The ERP results can be used to help interpret the mechanisms underlying these findings. The startle-elicited P300 is most commonly attenuated while viewing valenced pictures, suggesting that greater attention is being paid to more arousing pictures when compared to affectively neutral pictures (Bernat et al., 2011). This increased attention results in cross-modal inhibition of processing the auditory startle probe when viewing affective pictures. The current results suggest that the unpleasant stimuli may not have captured participants’ attention more than the neutral pictures. To our knowledge, this is the first study to measure startle responses using ERPs in healthy adolescents during an affective picture-viewing task. Thus, based on the current findings, it seems that early adolescents do not experience greater startle when viewing unpleasant images, although these findings require replication in this age group.

Overall, these findings related to startle-elicited responses are

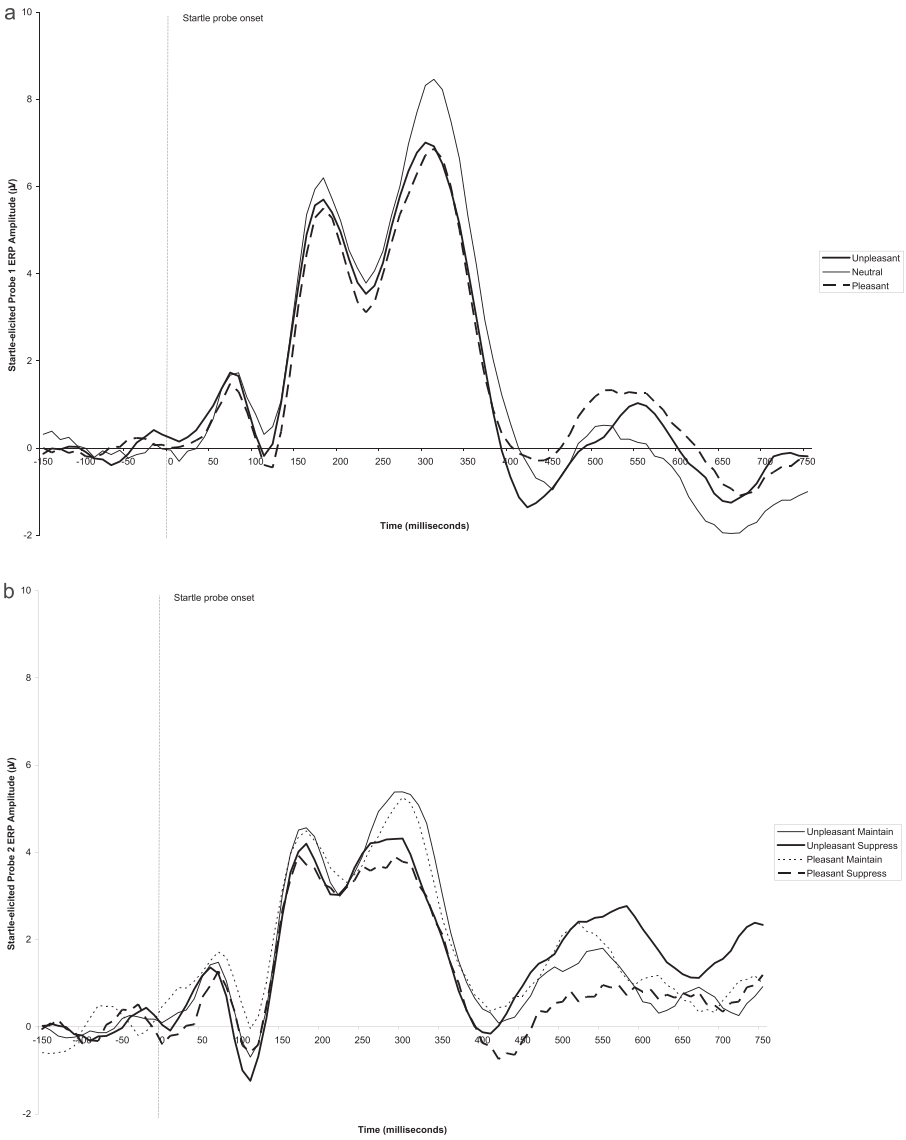

Fig. 3. Startle-elicited P300 amplitude at Pz during (a) emotional reactivity (top panel); and (b) emotional regulation (bottom panel).

Table 2
Means (standard deviations) in microvolts for raw startle blink EMG regulation by affective condition, instructions, and gender.

Affective Stimuli Instruction	Males	Females	All
Pleasant			
Suppress	111.01 (145.27)	115.59 (156.55)	113.24 (150.19)
Maintain	144.63 (179.58)	145.62 (190.89)	145.11 (184.32)
Neutral			
Maintain	122.77 (155.74)	144.99 (211.60)	133.58 (184.52)
Unpleasant			
Suppress	119.79 (163.96)	108.57 (157.99)	114.33 (160.45)
Maintain	138.70 (188.74)	137.25 (202.12)	138.00 (194.47)

consistent with other studies that have employed picture viewing paradigms with younger samples. The ERP data suggests that our participants' attention was greater for the pleasant than for neutral and unpleasant stimuli, which is also somewhat consistent with the lack of fear-potentiated startle reflex. However, this finding is not consistent with observed patterns of facial muscle activity or arousal measured through skin conductance. This suggests that this sample is attending and is likely to be responding with negative affect to the unpleasant stimuli, but is not experiencing the fear-potentiated startle reaction typically seen in adults. Although our findings cannot answer this question, they suggest that the difference we see in emotional reactivity to images between adolescents and adults may lie in the development of the startle response itself rather than being due to the lack of an affective response to the stimuli.

Given the number of studies, including ours, that have failed to find clear evidence of fear-potentiated startle in children and early adolescents during picture viewing, we speculate that the strength of this effect may not emerge until later in development, perhaps at some point during adolescence or early adulthood. Across species, the amygdala has been shown to be a key structure in fear-potentiated startle (Davis, 2006), and is also a brain region that undergoes significant developmental change during adolescence, in both volume and connectivity (Gabard-Durnam et al., 2014; Østby et al., 2009). It is therefore possible that the limited display of fear-potentiated startle in child and early adolescent samples reflects some immaturity of the amygdala or related brain structures involved in startle modulation. Schmitz et al. (2014) provide evidence that this startle mechanism develops along a pubertal,

rather than chronological, timeline. An important next step of work assessing emotional reactivity in adolescents would be to assess emotional reactivity while also examining brain, and specifically amygdala, development.

Alternatively, negative stimuli typically used in child and adolescent studies are rated on average less negatively, and as less arousing, than stimuli used in adult studies. It is possible that this explains the lack of fear-potentiated startle found in most child and adolescent samples. However, McManis et al. (2001) showed the same stimuli to children and adults and found differing patterns in their startle blink reactions. Our facial EMG and skin conductance data suggest negative emotions and high arousal when viewing the unpleasant images. Future studies should test whether varied levels of valence have differing impacts on the multiple physiological measures used here.

It is important to note that our findings only generalize to picture-viewing paradigms. Studies utilizing other threat paradigms, such as viewing movie clips (Quevedo et al., 2009) or blasts of air (Schmitz et al., 2014) have found fear-potentiated startle in younger samples. Thus, it is possible that early adolescents would display potentiated startle blink during aversive foreground stimuli in those types of paradigms. The goal of this study was to describe the physiological correlates of emotional reactivity in a specific paradigm; however, it is clear that future studies should utilize alternate, more intense startle-eliciting paradigms with early adolescents in order to further contribute to the research on this topic.

4.2. Emotional regulation

As stated above, we know of no study that has assessed the physiology of emotion regulation in early adolescents with the array of measurements used in the current study. Thus, the pattern of findings reported here are mostly novel and require replication. Following instructions to “stop” their reactions to the valenced stimuli, participants displayed opposite patterns in their facial muscle actions when compared to the “continue” condition. Specifically, corrugator activity was attenuated for unpleasant stimuli, and potentiated for pleasant stimuli during regulation. Although the effect of instructions on zygomatic activity did not reach statistical significance, inspection of Fig. 1 indicates that there was a pattern of reaction consistent with participants suppressing smiling facial expressions when asked to “stop” their emotional reaction while viewing pleasant stimuli. Presumably the high degree of variance in responses rendered this pattern difficult to detect with statistical tests, and future more highly powered studies may be needed to confirm this pattern of response.

Startle-blink magnitude was attenuated in the suppression condition for both pleasant and unpleasant pictures compared to neutral, and ERPs were also attenuated in the suppression condition compared to the maintain condition. Participants displayed lower skin conductance during emotion regulation than when they were maintaining their response to the valenced stimuli. This finding is particularly interesting given that adults’ skin conductance in previous studies has been observed to increase during “suppression” conditions (Gross, 1998), presumably because they are effortfully controlling their emotions and are, thus, more sympathetically aroused.

One possible explanation for this pattern of attenuated physiology during emotion regulation is that instead of effortfully regulating their emotions, early adolescents may have ignored the stimuli altogether when asked to stop their emotional response (despite our instructions to the contrary). This would theoretically lead to decreased skin conductance, since attention would not be directed to the arousing stimulus, nor to one’s own emotional response. However, if participants were disengaged from the stimuli, they would most likely have potentiated P300 ERPs since there would be no cross-modal inhibition. Participants instead showed attenuated ERPs in the suppression condition, which lends more support to the hypothesis that their focus is on effortful regulation. Thus, the pattern of skin conductance seen in this sample is one that particularly merits replication by future studies.

4.3. Limitations and strengths

This study had several limitations, which should encourage some caution in interpreting the findings. First, this study was cross-sectional and only assessed physiological correlates in 12-year-olds, so claims cannot be made about a developmental pattern arising, even though we know that some of these patterns look different in older samples. In addition, since this was a within-subjects study, there was no true control condition for emotion regulation. Rather, we were interested in the differences between regulating and maintaining. Interpretations must take this distinction into account. Also, participants knew they would be asked to regulate their emotions on a subset of trials. It is possible that this type of paradigm affected participants’ reactivity, preventing us from seeing an accurate range or intensity.

Emotion regulation was also confounded with time within each trial. However, similar paradigms have been used in adult studies measuring both reactivity and regulation (Dillon & Labar, 2005; Jackson et al., 2000). Participants in these studies displayed normative affective modulation of startle blink before regulation instructions, indicating that the paradigm does not compromise this affective pattern in adults. Startle probes only occurred at two presentation times – 2.5 s and 6 s after the presentation of the image. It is possible, although we feel unlikely given the number of trials and the difficulty in tracking these patterns, that participants were able to predict when the startle probe would be presented. If this type of prediction did occur, it could have altered startle responses, possibly affecting the modulation of startle by foreground stimuli.

The exploratory nature of the ERP data for emotional reactivity as well as all of the emotion regulation data also limits interpretation of the data until it has been replicated. Another important limitation was the low rate of scoreable startle responses in the current study. The rate obtained in this study was lower than is typical in other studies, including those from our laboratory. The most likely reason for the high rate of non-scoreable startle responses is movement artefact, which is known to be higher in younger samples (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). Greater attention to reducing movement artefact may be especially important in future studies with this age group.

The study also had important strengths. We used multiple physiological assessments that have the capability of providing separate pieces of information to create a cohesive picture of the mechanisms by which adolescents react to and regulate emotion. No studies to our knowledge in the child and adolescent literature have used such a comprehensive set of assessments. Additionally, our examination of physiological correlates of emotion regulation in adolescents is novel and, although the data must be interpreted with caution, it provides new information in a mostly unexplored field.

The current study provides information about emotional reactivity and regulation in adolescents. Given the absence of fear-potentiated startle at age 12 in the midst of expected facial muscle activity and skin conductance responses, there is evidence of potential development after early adolescence of the physiological and neural systems that control emotional reactivity, specifically as it relates to startle response. At this age, we see a pattern of physiology that suggests that early adolescents are effortfully employing emotion regulation strategies. However, our skin conductance findings are contradictory to that hypothesis, further warranting replication. Future research should also focus on the brain development specifically related to startle tasks, further informing the mechanisms underlying emotional reactivity in this age group. The current study provides a promising foundation for continued work in this surprisingly unexplored domain. Given the dramatic rise in the incidence of mental and behavioral disorders that have strong emotion symptomatology during the teenage years (Paus, Keshavan, & Giedd, 2008), understanding the individual differences and developmental

changes in these processes during early adolescence may provide new insights that can ultimately guide prevention and early intervention.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biopsycho.2017.07.018.

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