



## Registered Reports

# Enhanced startle reflexivity during presentation of visual nurture cues in young adults who experienced parental divorce in early childhood



Xenia Hengesch<sup>a,\*</sup>, Mauro F. Larra<sup>a</sup>, Johannes B. Finke<sup>a</sup>, Terry D. Blumenthal<sup>b</sup>,  
Hartmut Schächinger<sup>a,\*</sup>

<sup>a</sup> University of Trier, Institute of Psychobiology, Department of Clinical Psychophysiology, Johanniterufer 15, 54290, Germany

<sup>b</sup> Department of Psychology, Wake Forest University, Winston-Salem, NC 27109, USA

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## ABSTRACT

Adverse childhood experiences (ACE) may influence stress and affective processing in adulthood. Animal and human studies show enhanced startle reflexivity in adult participants with ACE. This study examined the impact of one of the most common ACE, parental divorce, on startle reflexivity in adulthood.

Affective modulation of acoustically-elicited startle eye blink was assessed in a group of 23 young adults with self-reported history of parental divorce, compared to an age- and sex-matched control group ( $n = 18$ ). Foreground pictures were either aversive (e.g. mutilation and injury), standard appetitive (e.g. erotic, recreational sport), or nurture pictures (e.g. related to early life, parental care), intermixed with neutral pictures (e.g. household objects), and organized in three valence blocks delivered in a balanced, pseudo-randomized sequence. During picture viewing startle eye blinks were elicited by binaural white noise bursts (50ms, 105 dB) via headphones and recorded at the left orbicularis oculi muscle via EMG.

A significant interaction of group  $\times$  picture valence ( $p = 0.01$ ) was observed. Contrast with controls revealed blunted startle responsiveness of the ACE group during presentation of aversive pictures, but enhanced startle during presentation of nurture-related pictures. No group differences were found during presentation of standard appetitive pictures. ACE participants rated nurture pictures as more arousing ( $p = 0.02$ ) than did control participants.

Results suggest that divorce in childhood led to altered affective context information processing in early adulthood. When exposed to unpleasant (vs. neutral) pictures participants with ACE showed less startle potentiation than controls. Nurture context, however, potentiated startle in ACE participants, suggesting visual cuing to activate protective behavioral responses.

## 1. Introduction

Adverse childhood events (ACE) are believed to induce negative long-term consequences, which may become evident decades after exposure. Physical and mental health problems tend to increase (Felitti et al., 1998; Wegman and Stetler, 2009), and especially stress-related psychological and cardiovascular disorders are shown to be more prevalent in adult participants who experienced ACE (McCauley et al., 1997; Batten et al., 2004).

Rodent models suggest that long-lasting changes in stress reactivity may link ACE to stress-related disease. Indeed, mother-pup separation in the neonatal period, as well as reduced maternal care behavior in early life, decreases the offsprings' physiological adaptive reactivity to stress episodes throughout later life (Plotsky and Meaney, 1993; Liu et al., 1997; Francis et al., 1999; McCormick et al., 2002).

However, human research has revealed conflicting results. ACEs have been found to induce hyper-responsiveness of the stress response systems, especially of the hypothalamus-pituitary-adrenal (HPA) axis (Heim et al., 2000), but other studies observed hypo-responsiveness and a suppression of the stress response to a psychosocial stressor in individuals maltreated in childhood (Carpenter et al., 2007, 2011). Such differences may be partially explained by variance in severity, onset, and duration of ACE (Heim et al., 2008). Different types of ACE have been characterized in humans, varying in nature and incidence. In an attempt to achieve a mostly homogenous sample we restricted recruitment to a single criterion, the experience of parental divorce in early childhood; this is the most common ACE (Rothman et al., 2008; Green et al., 2010; McLaughlin et al., 2010). Indeed, separation of parents and child has been found to enhance the risk of developing a psychological disorder (Kendler et al., 1992; Neher and Short, 1998;

\* Corresponding authors.

E-mail addresses: [hengesch@uni-trier.de](mailto:hengesch@uni-trier.de) (X. Hengesch), [schaechi@uni-trier.de](mailto:schaechi@uni-trier.de) (H. Schächinger).

Kristjansson et al., 2009) even when separation was only temporary (Raikkonen et al., 2011). Separation due to parental divorce has a similar effect on mental health (Hallstrom, 1987; Chase-Lansdale et al., 1995), especially when the divorce occurs before the child's age of 9 years. A recent study reports high negative long-term impact of divorce on adult health with the exposure aged between 0 and 7 years (Thomas and Hognas, 2015). Although the evidence suggests mild alterations in HPA axis activity of young adults from divorced parents (Bloch et al., 2007), neuroendocrine reactivity to a social stressor was found to be significantly lower in participants from divorced families compared to those with married parents (Kraft and Luecken, 2009).

Limbic brain structures such as the hippocampus and the amygdala may be involved in the long-lasting influence of ACE on stress responsiveness. These structures play an important role in the initiation and processing of stress responses. The amygdala appears to orchestrate the stress response (Lang and Davis, 2006; Ulrich-Lai and Herman, 2009), while stress hormone feedback on the hippocampus inhibits and terminates the neuroendocrine stress response (Tsigos and Chrousos, 2002). Indeed, a reduced hippocampal volume has been observed in depressed participants with childhood abuse compared to depressed non-abused individuals (Vythilingam et al., 2002) and non-depressed control participants (Saleh et al., 2017), while other studies observed an increased amygdala volume in human participants who experienced ACE (Buss et al., 2012; Pechtel et al., 2014). Given the fundamental role of the limbic system in emotional and memory processing, it is not surprising that ACE influence a variety of cognitive functions and behaviors. Indeed, memory deficits have been found in adult human survivors of childhood abuse (Bremner et al., 1995), as well as in rodents experiencing early maternal deprivation (Oomen et al., 2011), effects presumably mediated by hippocampal impairment. Amygdala over-activity (van Harmelen et al., 2013) and information-processing biases for facial displays of emotion (Gibb et al., 2009) have been found in human adults reporting ACE.

ACE-induced over-activity of the amygdala may also lead to changes in startle responsiveness. However, only a few studies have addressed this research topic. The startle reflex is a physiological response to a sudden and unexpected stimulus (Davis, 1984; Koch, 1999). It consists of several components, such as somatic and facial muscle responses (e.g. eye blink), autonomic nervous system activation (e.g. heart rate and electrodermal responses), endocrine responses (e.g. HPA axis activation), and behavioral changes (e.g. acceleration of response times). Responses are orchestrated by the amygdala and prepare the organism for rapid action, e.g. fight and flight (Lang and Davis, 2006). The startle eye blink is the most sensitive and consistent startle response across human individuals and can be easily and reliably elicited in laboratory settings by the presentations of abrupt and intense acoustic stimuli (Koch, 1999).

There is evidence that previous stress episodes may influence the startle reflex. Several studies have shown startle hyper-responsiveness in patients suffering from posttraumatic stress disorder (PTSD) (Ornitz and Pynoos, 1989; Morgan et al., 1997; Metzger et al., 1999; Lipschitz et al., 2005). Similarly, enhanced startle has also been found in women with self-reported physical or sexual abuse in early childhood, suggesting a long-lasting sensitization of the startle eye blink reflex due to the experience of ACE (Jovanovic et al., 2009). However, it remains unclear whether startle enhancement in ACE participants is the result of a per se increased startle reflex, or whether participants' actual emotional processing is leading to a contextually-driven startle potentiation.

An ideal experimental model to clarify this question is offered by the "affective startle modulation paradigm" (Lang et al., 1990). The cognitive mechanism underlying affective startle modulation is best explained by motivational priming, with the emotional context in which startle is elicited influencing the magnitude of the startle response. Given an aversive emotional context the defensive motivational system is primed to facilitate the processing of defensive reflexes, such as the startle reflex. Given an appetitive emotional context the reflex is

inhibited. Many studies, including our own (Lass-Hennemann et al., 2010; Ferreira de Sá et al., 2014), have shown that an individual's emotional state may lead to either startle potentiation or attenuation, irrespective of the sensory modality by which the emotional state was induced: pictures (Bradley et al., 1990), films (Kaviani et al., 1999) and music of positive or negative hedonic valence (Roy et al., 2009). However, most often visual stimuli, such as pictures included in the International Affective Picture System (Lang et al., 2008), are used. We used this type of foreground stimuli as well; however, since we were interested in an early childhood/nurture context, we additionally included high quality pictures of laughing babies, mother/child interactions, and nurture-related objects (e.g. pacifiers). Most often, affective startle modulation is examined by randomized presentation of affective foreground stimuli intermixed with neutral pictures. We were concerned about carry-over effects between adjacent pictures – especially in the sequence of either erotic or violence presentations followed by nurture/baby cues or vice versa – which may create a socially unacceptable association that might disturb participants' willingness to participate and/or tending to induce unfavorable response biases. To avoid such carry-over effects a special block design was created. Affective pictures of three different qualities (negative valence, positive valence, and nurture-related) were presented in three different blocks of a single valence intermixed with non-emotional, neutral stimuli (e.g. household objects) serving as a neutral reference within each block. Separating valence by block but with neutral fillers (Bradley et al., 1996) removes carry-over effects. Similar designs have been successfully used before and have proved to be feasible (Schlam et al., 2011). Thus, the design used in the current study allowed us to examine the impact of nurture cues vs. neutral cues, and to compare the effects with the results found after presentation of general affective stimuli.

## 2. Methods

### 2.1. Participants

Forty-nine undergraduate students with or without history of parental divorce were recruited by announcements published at the University of Trier and the Trier University of Applied Sciences. Exclusion criteria were acute or persistent medical and psychiatric diseases, current medication except the occasional use of pain killers (paracetamol, aspirin, or NSAR), heavy smoking (> 10 cigarettes per day), regular drinking of alcohol beverages (> 30 g/day), illicit drug intake within the last 6 months, current or past hearing problems (e.g. tinnitus), or presence of any ACE other than parental divorce. Presence of childhood abuse was checked beforehand by an interview and the *Childhood Trauma Questionnaire* (CTQ; see below) and participants were excluded in case of sexual and/or physical abuse.

All study procedures were approved by the local ethical committee and participants gave written informed consent prior to study participation. Financial compensation was 20 €. Eight participants (3 with parental divorce, 5 without parental divorce) were later excluded from statistical analysis because of sexual and physical abuse (1 participant), physical abuse (1 participant) and complete habituation of startle eye blink (referred to as "startle non-responders": participants that fail to respond to the startle noise probe, with no detectable startle eye blink response in 10 consecutive trials, 6 participants). The final sample consisted of 23 women and 18 men, of which 23 participants belonged to the ACE group (ACE; 13 women). Volunteers who were themselves parents were not included. Participants of the control group (CNT; 11 women) had non-divorced parents through the time of testing, and no other childhood trauma.

### 2.2. Procedures

Experimental sessions started in the afternoon between 1 and 5 pm with an interview screening session. Participants were then asked to fill

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