



## The conditioning and extinction of fear in youths: What's sex got to do with it?



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### ABSTRACT

Adult work shows differences in emotional processing influenced by sexes of both the viewer and expresser of facial expressions. We investigated this in 120 healthy youths (57 boys; 10–17 years old) randomly assigned to fear conditioning and extinction tasks using either neutral male or female faces as the conditioned threat and safety cues, and a fearful face paired with a shrieking scream as the unconditioned stimulus. Fear ratings and skin conductance responses (SCRs) were assessed. Male faces triggered increased fear ratings in all participants during conditioning and extinction. Greater differential SCRs were observed in boys viewing male faces and in girls viewing female faces during conditioning. During extinction, differential SCR findings remained significant in boys viewing male faces. Our findings demonstrate how sex of participant and sex of target interact to shape fear responses in youths, and how the type of measure may lead to distinct profiles of fear responses.

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### 1. Introduction

A wealth of work performed in adults reports that sex may influence emotional processing. Not only are there differences in the way men and women recognize and process emotions, there are also differences in the way the sex of facial targets used in tasks may modulate the analysis of emotional cues (Kret & De Gelder, 2012; Kring & Gordon, 1998; Pattwell, Lee, & Casey, 2013). Little is known about the influence sexes of participants and targets may have on emotional processing in younger population. In the current study, we aim at better understanding the influence of sex of participant and sex of target on fear conditioning and extinction in youths, as these tasks are often used to study emotions and emotion-related brain function in healthy and psychiatric paediatric populations.

Fear conditioning refers to the process by which a neutral stimulus is paired with an aversive unconditional stimulus (US; e.g. electric shocks), becoming a conditioned stimulus (CS+) eliciting a conditioned fear response when presented independently of the US. In humans, this response is usually measured with subjective fear ratings and/or skin conductance responses (SCR) (Lissek et al., 2005). Fear extinction, in comparison, refers to the diminution of the fear response after repeated presentation of the CS+ without the US. In discrimination fear conditioning and extinction, fear responses to the CS+ are compared to fear responses to a CS never paired with the US (CS−), which serves as a safety signal.

When using such classical fear conditioning and extinction paradigms in rodents, males show greater fear conditioning and more resistance to fear extinction than females, differences that emerge around puberty, presumably due to effects of sex hormones (Dalla & Shors, 2009). In human youths, no prior work examined sex differences during fear conditioning and extinction; however, tasks using intrinsically evocative faces were employed. Findings from this work report mixed observations. An important amount of studies demonstrated that female children and adolescents are more negatively aroused by threatening faces, as well as faster and

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more accurate in labelling and recognizing these cues, compared to male children and adolescents (Lee et al., 2013; see reviews by Kret & De Gelder, 2012; McClure, 2000). Some other work, however, did not observe sex differences in emotional processing, neither in child nor adolescent samples (De Sonnevile et al., 2002; Herba, Landau, Russell, Ecker, & Phillips, 2006; Kret & De Gelder, 2012; McClure, 2000; Thomas, De Bellis, Graham, & LaBar, 2007; Vicari, Reilly, Pasqualetti, Vizzotto, & Caltagirone, 2000). In adults, conflicting findings are also observed. Women are shown to rate the CS+ and the US as more distressing and unpleasant than men during fear conditioning and extinction (Forsyth & Eifert, 1998; Kelly & Forsyth, 2007). Increased fear ratings of pain during movement-related conditioning are also observed in women relative to men (Meulders, Vansteenwegen, & Vlaeyen, 2012). When using physiological responses (SCRs, brain activation), however, men have often been reported as more physiologically reactive during the processing of threatening stimuli – especially male facial cues – compared to women. These findings were interpreted in an evolutionary perspective, with men prepping defence responses towards other threatening rival males, in relation with reproduction and survival (Kret & De Gelder, 2012; Milad et al., 2006). Regarding fear conditioning specifically, physiological data are less clear-cut as some findings show greater SCRs to facial threat cues in men relative to women (Dimberg & Öhman, 1996), whereas other work report similar SCRs to facial threat cues in both men and women (Kret & De Gelder, 2012; Navarrete et al., 2009).

Concerning the influence sex of facial targets may have on emotional processing, threatening male facial expressions (anger, fear) have consistently been demonstrated to activate greater fearful responses than threatening female facial expressions. This was observed in both youths and adults (Aguado, Garcia-Gutierrez, & Serrano-Pedraza, 2009; Becker, Kenrick, Neuberg, Blackwell, & Smith, 2007; Egger et al., 2011; Goos & Silverman, 2002; Hess, Blairy, & Kleck, 1997; Navarrete et al., 2009; Seidel, Habel, Kirschner, Gur, & Derntl, 2010).

In the current study, we aimed at examining, firstly, the influence of sex of participants, and secondly, the influence of sex of target, on fear learning and extinction in boys and girls aged 10–17 years old. To reach this goal, we used a discrimination fear conditioning and extinction paradigm recently developed by Lau and collaborators (Lau et al., 2008, 2011). This unique paradigm uses a paediatrically safe US shown to be as efficient as the US usually employed in animals and adults, electric shocks, which may not be used in youths due to ethical considerations. Head shots of two different actresses constitute the CS+ and CS– (neutral facial expressions) and the US is constituted of the CS+ actress's picture depicting a fearful facial expression, which is simultaneously presented with a shrieking female scream. Hence, here, we capitalized on the intrinsic aversiveness of witnessing fear in others. With this task, Lau and collaborators were successful in triggering fear acquisition and extinction, as measured through fear ratings and SCRs in healthy and anxious youths (Lau et al., 2008, 2011). However, the influence of sexes of participants and targets was not measured in these previous studies.

Taking these two variables into account, and based on the above mentioned findings (especially those concerning human youths), we hypothesized that during conditioning, (1) boys and girls would show differential learning, manifested as greater fear evoked by the CS+ vs. CS–, (2) girls would show greater overall fear responses (CS+ and CS–) compared to boys, and (3) male fearful facial expressions would trigger greater fear responses in both sexes compared to female faces. During extinction, both boys and girls should extinguish fear, with levels of fear responses being similar for both the CS+ and CS–. Overall fear responses (CS+ and CS–) should remain higher in girls compared to boys, and for male faces relative to female faces in both sexes.

## 2. Method

### 2.1. Participants

A total of 120 healthy participants completed the study. Participants ranged in age from 10 to 17 years. Exclusion criteria for participation in the study were any type of past or present mental disorders, medical illness and use of medication as assessed by self-report in youths and one of their parents. Subjects were recruited in community centres (e.g., libraries, day camps) as well as schools of the Montreal greater area using flyers. The study protocol was approved by the Research Ethics Boards of the CHU Ste-Justine, Montreal, Canada. Participants and their parents gave informed assent and consent, respectively, and youths were compensated for their participation. Of the initial 120 participants recruited, two abandoned before completing the study, and data for one participant were lost due to technical problems. Hence, analyses were carried on 117 youths, 56 boys (mean age =  $14.05 \pm 2.11$ ) and 61 girls (mean age =  $13.77 \pm 1.93$ ).

### 2.2. Experimental design

The paradigm lasted 17 min and comprised two phases: a fear conditioning phase and a fear extinction phase (Lau et al., 2008, 2011). During each phase, participants saw head shots of individuals presenting neutral emotional expressions. These photos were selected from the NimStim Set of Facial Expressions (Tottenham et al., 2009). One individual was randomly selected to serve as the conditioned stimulus (CS+) for each participant, whereas the other served as the CS– (safety signal). During conditioning, the CS+ was paired with the US on 50% of trials. Because fear conditioning is a process inducing a fear response that tends to naturally decrease over time, a partial reinforcement contingency ratio was used to prevent habituation to the US (Mackintosh, 1974). The US was constituted of the photo of the same actor/actress selected for the CS+, but depicting a fearful expression and presented simultaneously with a 90 dB shrieking male or female scream. Participants were not aware of the CS+ – US association prior to the experiment. The other actor/actress served as a conditioned stimulus unpaired (CS–) with the aversive US. In the present study, 49 participants (24 boys, 25 girls) saw photos of males posing as the CS+ and CS–, while 68 participants (32 boys, 36 girls) saw photos of females posing as the CS+ and CS–. Four groups were constituted: Group 1 – boys viewing male facial expressions; Group 2 – girls viewing male facial expressions; Group 3 – boys viewing female facial expressions; Group 4 – girls viewing female facial expressions. During extinction, task procedures were identical to that of the conditioning session except that no US were presented, and only 14 CS+ unpaired and 14 CS– were shown.

Fear ratings and SCRs served as the behavioural dependent measures, i.e., the fear responses to the CS+ and CS– during conditioning and extinction. Fear ratings were performed during each presentation of the photographs (CS+ before apparition of US, CS–), in both the conditioning and extinction phases (Fig. 1). Participants were asked to indicate on a 5-point Likert scale the degree to which they felt afraid when viewing the actor/actress in the CS+ and CS– photos (Are you afraid?; 1 = not at all, 5 = extremely). Fear ratings were recorded with a right hand-held button response box developed to allow for a graded range of responses (Current designs, Philadelphia).

Overall, 84 stimuli were presented (Fig. 1). Conditioning trials ( $n = 56$ ) comprised one of the three events: CS+ paired ( $n = 14$ ), CS+ unpaired ( $n = 14$ ) or CS– stimuli ( $n = 28$ ). The CS+ paired events consisted of the presentation of a neutral face stimulus (3-s), a rating response (3-s), and a fearful face stimulus (1.1-s) paired with the auditory stimulus (1-s). The CS+ unpaired and CS– events consisted of the presentation of the neutral face stimulus (3-s), followed by the rating response (3-s). Events were presented for durations of 6 (CS+ unpaired and CS–) or 7.1 (CS+ unpaired) s with inter-stimulus intervals of 3, 4, 5, 6, 8, 10 or 12 s. During extinction, 14 CS+ unpaired (3 s – face presentation, 3 s – rating response) and 14 CS– (3 s – face presentation, 3 s – rating response) were shown. Trials were presented in a pseudorandom order and the assignment of actors or actresses (either blond or brown hair) to CS-type (CS+, CS–) was counterbalanced across participants.

Before testing, participants were familiarized with the discrimination conditioning and extinction tasks to ensure understanding of picture rating. The pictures presented during the practice session were different from the ones used during the actual fear conditioning and extinction paradigm to prevent habituation to the CS+, US and CS–. Before practice and testing sessions, participants were told they would see two different images and hear sounds, but no details were given on the images or sounds. Visual and auditory stimuli were presented through a laptop computer using E-Prime software (PST, Inc., Pittsburgh, PA) and headphones were placed on the ears of the participants.

Following completion of the conditioning and extinction tasks, photos of the actors or actresses used for the CS+ and CS–, and depicting a neutral facial expression, were presented again to participants, who were asked to rate their fear levels on the 5-point Likert scale one last time. During this post-experiment interview, participants were also debriefed and asked about their contingency awareness of the CS–US relationship. Specifically, youths were asked if the blond- and/or brown-haired actor/actress screamed. Contingency awareness (1 = yes, 0 = no) was granted if participants correctly identified which actor/actress had been paired with the scream (CS+), and which represented the safety signal (CS–).

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