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Chemosensory anxiety signals prime defensive behavior in prepubertal girls

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HIGHLIGHTS

- Chemosensory anxiety signals prime withdrawal-related motor responses in adults
- The reproductive status affects the perception of chemosignals in children.
- We examine prepubertal girls' startle response in the context of anxiety chemosignals.
- Girls show pronounced startle amplitudes during exposure to anxiety chemosignals.
- Human anxiety chemosignals are functional irrespective of reproductive status.

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ABSTRACT

Chemosensory anxiety signals effectively prime motor responses related to withdrawal behavior, such as the startle reflex, in adult humans. As the reproductive status strongly affects the response to social chemosignals, the current study examined whether chemosensory anxiety signals would augment the startle response in prepubertal children as it does in adults.

Using cotton pads, axillary sweat was collected from 28 men while waiting for an important oral examination (anxiety condition), and during ergometer training (sport control condition). Using a constant-flow olfactometer, sweat samples and pure cotton samples (cotton control) were presented to 10 prepubertal girls aged 9–13 years ($M = 11.25$, $SD = 1.25$) for 3000 ms during inhalation. White noise bursts of 102 dB(A) served as startle probes, and startle responses were recorded via electromyography of the orbicularis oculi muscle.

The girls showed larger startle amplitudes to probes presented in the context of chemosensory anxiety signals as compared to a context of sport control sweat ($p < 0.01$) as well as cotton control ($p < 0.05$). This effect was not attributable to differences in stimulus detection rates or stimulus hedonics.

The results show that in prepubertal girls, similar to adults, chemosensory anxiety signals prime defensive motor behavior. This effect appears unrelated to the odorous quality of anxiety sweat, but seems to reflect a specific preparedness to respond to the underlying social alarm signal. Thus, chemosensory communication supporting individual harm protection is independent of the reproductive status in humans.

1. Introduction

The perception of chemical compounds from human bodily secretions is largely affected by sex hormonal status. In adults, the perception of steroidal body odor compounds is correlated to the level of free testosterone and estradiol [1]. Further, infants and children are generally more sensitive to these compounds when compared to adults [2,3], but with puberty the sensitivity of boys decreases while that of girls increases [4,5]. Moreover, while boys, after entering puberty, display a reduced aversion towards their fathers' body odor [6], a mutual aversion of the other's body odor emerges in father-daughter relationships when daughters reach puberty [7]. Thus, sex hormone-dependent volatiles in body odor affect interactions within the family circle, and in contexts related to affiliation and reproduction.

However, other chemocommunicative pathways, such as basic mechanisms related to harm protection, should be independent of the sex hormonal status [8]. For example, stress-related chemosignals conveyed in body fluids prime the fight–flight-response in adult human perceivers, as indicated by an augmented startle reflex during exposure to anxiety sweat [9,10]. Likewise, adult rodents show an increased

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startle response in the context of chemosensory stress signals emitted by conspecifics [11]. These animal data argue in favor of a mechanism that appears evolutionarily conserved across species. Here, we enquire whether stress-related chemosignals are effective during ontogeny.

The current study aimed at investigating children's withdrawal-related motor behavior in the context of adult chemosensory anxiety signals. In order to assess the prepubertal status most effectively in a first step, only pre-menarcheal girls were investigated. It is expected that, unlike girls' altering response to body odorants related to reproduction, the reception of chemosensory anxiety signals should not differ across puberty. In other words, the auditory startle response of prepubertal girls should be augmented when primed by anxiety chemosignals, similar to what has been shown with adults.

2. Methods

2.1. Participants

Participants were 10 girls aged 9–13 years (M = 11.3, SD = 1.3), who had not reached menarche. These girls were non-smokers, did not suffer from any physical illnesses, especially not from diseases of the upper respiratory tract or the auditory system (self-report), and were neither on acute nor on any long-term medication. Moreover, none of the girls suffered from any internalizing or externalizing behavioral problems [12]. In order to screen for general hyposmia, the girls were required to identify a bottle containing phenyl-ethyl alcohol (99%, Fluka, Germany, 1:200 (v/v) diluted in 1,2-propanediol) from a set of three bottles in two consecutive trials, with the remaining two bottles containing the same volume of propanediol. Phenyl-ethyl alcohol was chosen as test odorant for general hyposmia since it is a purely olfactory odor used as a standard in olfactory sensitivity testing [13], and to date no case of specific anosmia to phenyl-ethyl alcohol has been reported [14]. No girl had to be excluded due to general hyposmia.

Written, informed consent was obtained from their parents, and the girls were financially compensated for their participation. The study was approved by the ethical committee of the Medical Faculty of the University of Kiel.

2.2. Chemosensory stimuli collection and presentation

Axillary sweat was collected via cotton pads from 28 male students while awaiting an oral examination at the university (anxiety signal). One chemosensory control stimulus was a sweat sample from the same individuals while participating in ergometer training (sport control). A second chemosensory control was the odor of unused cotton pads (cotton control), treated in exactly the same way as both body odor samples. The donors felt more anxious (visual analog scale "anxiety", $t(27) = 16.30$, $p < 0.001$), less happy (Self-Assessment Manikin, SAM [15]: valence, $t(27) = 6.14$, $p < 0.001$) and more submissive (SAM: dominance, $t(27) = 5.17$, $p < 0.001$) during the anxiety compared to the sport condition. Arousal did not differ between conditions (SAM: arousal, $p = 0.14$, all p-values are Bonferroni corrected). Both salivary cortisol and testosterone increased during the anxiety condition, and decreased during the ergometer condition (cortisol: $F(2, 34) = 14.81$, $p < 0.001$, testosterone: $F(2, 32) = 3.98$, $p = 0.04$). More details on the donors, the sweat sampling procedure and the assessment of hormones can be found elsewhere [16]. Prior to presentation, the sweat samples were pooled across donors with respect to the given chemosensory condition. The sweat samples were presented according to Kobal [17], using a constant-flow (100 ml/s; stimulus duration = 3 s), six channel olfactometer (OM6b, Burghart, Wedel, Germany). Both nostrils were stimulated simultaneously with an air flow of 37 °C (humidity above 80%).

2.3. Startle probe

The startle-eliciting stimulus was a 102-dB (A) white noise burst (duration = 50 ms, rise-time < 1 ms), presented via insert earphones (ER3-14A, Etymotic Research, Inc., IL, USA).

2.4. Stimulus detection and ratings

To determine participants' detection performance of anxiety signal and sport control, the chemosensory stimuli were administered via the olfactometer (duration = 3 s). The participants were asked to select the most intense stimulus from a series of three stimuli (three-alternative forced choice, including one worn cotton pad, either from the anxiety or from the sport condition, and two blank odors consisting of clean cotton pad). This procedure was carried out twice for each chemosensory conditions. Participants who failed once to detect the chemosensory stimulus (from the anxiety or the sport condition) were considered not to be able to smell the respective odor. Intensity, pleasantness, unpleasantness, and familiarity ratings were assessed using visual analog scales presented on a computer screen. In addition, the participants were asked to identify the emotional state of the sweat donors. Following each odor presentation during the eye blink recording, the six basic emotions were depicted on a computer screen as facial expressions of cartoon characters, and the participants indicated their guess by mouse click (forced choice).

2.5. Procedure

All participants were tested individually, without the presence of the experimenter or parents. The room temperature was kept between 19 °C and 21 °C. At the beginning of the sessions, the olfactory hyposmia screening and the stimulus detection test were carried out, followed by the stimulus ratings. After a short break, the eye blink response was recorded in the context of the chemosensory stimuli donated during the anxiety condition, the sport control condition, and the cotton control condition. The chemosensory stimuli were presented in pseudo-randomized order within two blocks of 27 trials each. Each stimulus was presented 18 times in total. Both blocks started with a habituation phase lasting 30 s, including the presentation of 9 startle probes [18]. At the beginning of each trial, a visual countdown instructed the participants to prepare for inhalation (duration = 3.6 s). During the subsequent inhalation phase (duration = 3 s), the chemosensory stimuli were presented (the odor valve was activated 0.6 s before the inhalation phase started). During each trial, two startle probes were presented (first: randomly between 2 s and 3 s after the beginning of the inhalation phase. Second: randomly between 9 s and 10 s after the end of the inhalation phase. The second probes served to prevent classical conditioning between the tone and the odor, thus the startle responses to the second probes during the inter-stimulus-interval were not analyzed.). Startle probes were embedded in a background white noise of 70 dB (A). The trial duration was 20 s. One second after the inhalation phase ended, the participants were asked to identify the emotional state of the donors within a period of 5.4 s. The participants' breathing cycle was recorded with a respiration belt attached to the thorax (Zak GmbH, Simmbach/Inn, Germany).

2.6. Data recording

The eye blink component of the startle reflex was recorded bipolarly from the *orbicularis oculi* muscle beneath the left eye [19], using Ag/AgCl electrodes (6 mm inner diameter). The ground electrode was placed on the forehead. Data were sampled at 2000 Hz, and filtered online using a 50-Hz notch filter. Data were recorded, amplified and filtered with Acquire 4.2 (NeuroScan Inc., Virginia, USA). The raw EMG was high- (30 Hz, 24 dB/octave) and low-pass filtered (500 Hz, 24 dB/octave, [20]).