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Positive Emotional Priming of Facial Affect Perception in Females is Diminished by Chemosensory Anxiety Signals

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Abstract

Chemosensory communication of anxiety is a common phenomenon in vertebrates and improves perceptual and responsive behaviour in the perceiver in order to optimize ontogenetic survival. A few rating studies reported a similar phenomenon in humans. Here, we investigated whether subliminal face perception changes in the context of chemosensory anxiety signals. Axillary sweat samples were taken from 12 males while they were waiting for an academic examination and while exercising ergometric training some days later. 16 subjects (eight females) participated in an emotional priming study, using happy, fearful and sad facial expressions as primes (11.7 ms) and neutral faces as targets (47 ms). The pooled chemosensory samples were presented before and during picture presentation (920 ms). In the context of chemosensory stimuli derived from sweat samples taken during the sport condition, subjects judged the targets significantly more positive when they were primed by a happy face than when they were primed by the negative facial expressions ($P = 0.02$). In the context of the chemosensory anxiety signals, the priming effect of the happy faces was diminished in females ($P = 0.02$), but not in males. It is discussed whether, in socially relevant ambiguous perceptual conditions, chemosensory signals have a processing advantage and dominate visual signals or whether fear signals in general have a stronger behavioural impact than positive signals.

Key words: anxiety, chemosensory communication, emotional priming, facial expression

Introduction

Socially relevant emotions like aggression (Rich and Hurst, 1998), anxiety and stress (von Frisch, 1941), or the reproductive state (Penn, 2002) are chemosensorily communicated in many vertebrates (see Wyatt, 2003). These signals evoke physiological and behavioural adaptations in the perceiver, fostering ontogenetic and probably phylogenetic survival also (see Hildebrand, 1995; Schaal *et al.*, 2003; for a critical review on pheromones in mammals, see Doty, 2003).

In mammals, transmission of chemosensory fear signals was first described by Valenta and Rigby (1968) using a rat model. Following studies with mice indicated that the perception of these pheromonal signals is transmitted via the olfactory system (Rottman and Snowdon, 1972). Rodents generally respond to chemosensory signals of anxious conspecifics with withdrawal behaviour (Carr *et al.*, 1970; Mackay-Sim and Laing, 1981; Zalaquett and Thiessen, 1991): they avoid the odour source and show increased motor activity (defensive action). However, if the odour donors are strongly stressed, the signal receivers respond with freezing-like behaviour (defensive immobility; Mackay-Sim and Laing, 1981). Additionally, it has been shown that the avoidance of chemosensory anxiety signals is reduced in those mice which were socially isolated after weaning (Rottman and Snowdon, 1972). The withdrawal behaviour is associated with vegetative alterations, such as hyperthermia (Kikusui *et al.*, 2001) and changes within the neuropeptide and immune system (Fanselow, 1985; Cocke *et al.*, 1993; Moynihan *et al.*, 2000). These vegetative changes are considered to reflect the classical stress-induced physiological adaptations (Fanselow, 1985; Moynihan *et al.*, 2000; Kikusui *et al.*, 2001).

So far, in humans, only a few studies examined whether emotional states can be communicated via chemosensory signals. Performing a subjective rating study, Owen (1981) investigated body odours from anxious, sexually aroused and relaxed subjects and concluded that anxiety might contribute most to the production of emotion-specific chemosignals. Chen and Haviland-Jones (2000) exposed male and female odour donors to funny and frightening movies, collected their axillary sweat and asked male and female participants to identify the emotions of the donors by smelling the sweat samples. The results indicate that fear odour could be better identified than the odour of happy people; however, this effect could only be observed when the body odour samples were taken from males and not from females. Moreover, females were found to show higher iden-

tification rates than males. In a third study, only females were recruited as odour donors and as perceivers (Ackerl *et al.*, 2002). Underarm sweat was sampled from women watching either a neutral or a horror film. The odour samples taken in the anxiety condition were judged to be more intense, less pleasant and more aggressive-like than the samples taken in the control condition. However, they were not described as fear-like.

The aim of the present study was to investigate whether chemical substances, taken from subjects during the experience of high state anxiety, modulate implicit social perception. In contrast to the aforementioned studies, we changed two main conditions. First, we did not induce anxiety by frightening movies. Instead, sweat samples were taken while students were waiting for their academic examination, in order to realize an ecologically valid emotional situation. Thereby, we expected the sweat donors to experience a high and not only a moderate (see Chen and Haviland-Jones, 2000; Ackerl *et al.*, 2002) degree of anxiety. Similarly, we expected anxiety, experienced during a meaningful und relevant situation, to be accompanied by arousal (see Bradley and Lang, 1994). An experience of arousal may be necessary in order to initiate some behavioural effects of stress hormones (e.g. adrenocorticotropin or cortisol; McGaugh, 2000; Buchanan and Lovallo, 2001). These stress hormones, in turn, are also considered to mediate the production of alarm chemosignals in rats (Abel, 1994).

Secondly, we expected the chemosensory effects to be processed implicitly rather than explicitly. The phenomenon that undetectable chemosensory stimuli have significant effects on the behaviour and physiology in humans has often been described (overview in Köster, 2002; Jellinek, 2003). Highly time-sensitive measures of brain activity, such as EEG methods, could reveal that brain activity changes during the presentation of undetectable odours (Lorig, 1989, 2000) and that body odours can be differentiated on a preattentive level (Pause *et al.*, 1999a,b). Additionally, undetectable putative pheromones activate specific brain regions in humans (Sobel *et al.*, 1999). Finally, strong evidence for pheromonal effects in humans has been provided by investigating nonodourous substances from the human axilla (Stern and McClintock, 1998).

Here, we have chosen a priming task based on the perception of facial expressions. It has been reliably documented that affective judgements can be changed by subliminal exposure to visual primes (Kunst-Wilson and Zajonc, 1980). Consecutive studies demonstrated that subliminally presented facial expressions successfully serve as biologically relevant primes (Murphy and Zajonc, 1993; overview in Öhman, 2004). For the present study, we adopted the priming design of Höschel and Irle (2001).

The subjects had to judge a neutral facial expression which was preceded by subliminal presentations of emotional facial expressions. The chemosensory stimuli were presented at the threshold level and implemented as context cues for the picture presentation. As the subjects were not aware of the primed nature of their judgements, an effect of the chemosensory stimuli on these judgements would demonstrate that chemosensory anxiety signals are implicitly processed as important biological signals.

We hypothesized that such signals should affect subliminal face perception, either by strengthening the perception of fearful faces or, more generally, of negative facial expressions, or by weakening the perception of happy faces. Thus, in either case, the percentage of negative judgements should be increased in the context of chemosensory anxiety signals. The unidirectional nature of our hypotheses is based on the fact, that all studies on the effects of chemical communication of anxiety in mammals indicate that the signal receivers show a stress-typical physiology and withdrawal behaviour.

Considering the results of the study from Chen and Haviland-Jones (2000), we collected the sweat samples only from male students and investigated the responses of male and female subjects separately. In order to avoid a confound of trait and state anxiety, we excluded sweat donors who were generally anxious. Similarly, we only investigated subjects who scored low on social anxiety (see Rottman and Snowdon, 1972).

Materials and methods

Participants

Twenty subjects reporting no history of chronic medication, of neurological, psychiatric, endocrine or immunological diseases, or of diseases related to the upper respiratory tract participated voluntarily in the experiment (due to these criteria, six subjects had to be excluded prior to the experiment). All participating subjects had to fill in a German questionnaire on social uncertainty (U-Fragebogen; Ullrich and Ullrich, 1977); two females who scored 1.5 standard deviations above the standardized mean for social anxiety (factor II) were excluded. As two further male subjects had to be excluded (one subject could identify the control face during the priming procedure, another subject was omitted due to technical problems during the picture presentation), 16 subjects (eight females) remained in the final sample and could be analysed. They had a mean age of 22.6 years (SD = 3.0; range = 19–29 years) and four of them described themselves as smokers. Seven females used oral contraceptives and the eighth stated that she had a regular menstrual cycle. All participants gave written, informed consent and were paid for participation. The study was conducted in accordance with the declaration of Helsinki.

Sweat donors and presentation of chemosensory stimuli

Fourteen male university students agreed to donate axillary sweat (collected on cotton pads). However, one donor was excluded because he did not fix the pads properly and another, because he scored too high in trait-anxiety [according to the State-Trait-Anxiety Inventory, STAI,

Laux *et al.* (1981), his degree of trait anxiety was in the upper 2% of the standard sample]. The remaining 12 donors indicated that they did not suffer from any medical disease, especially not from a metabolic disease, nor did they chronically use medication. They were on average 25.4 years old (SD = 4.7, range = 20–38 years); two described themselves as smokers. As all donors participated in the anxiety as well as in the sport condition (see below); a possible effect of smoking on body odour was balanced between the conditions.

The donors had to collect their sweat from both axillae for a duration of 1.5 h in two conditions (steroid stress hormones are secreted with a relatively long latency of 20–30 min; accordingly, we collected the sweat for a relatively long period). The first situation (anxiety condition) took place at the university and was an oral examination to obtain an academic degree (eight bachelor, four master examinations in psychology, medicine or pharmacology). The cotton pad was fixed while the students were waiting for their examination and removed immediately before the examination started. During the second, control condition, the donors were asked to exercise on a bicycle ergometer (three times for 10 min with a constant heart rate of 110, separated by 10 min breaks). The pads were fixed 30 min before the sport session started and removed after the 1 h long session. The control condition was carried out on average 8.5 days (SD = 6.3) after the anxiety condition; the time of day of both situations was held constant within subjects (range 0.5 h). The donors were asked to refrain from using deodorants and to wash their armpits exclusively with water 1 day before the sweat was collected. Furthermore, they were requested to refrain from eating onions, garlic or asparagus. All donors participated voluntarily and gave written, informed consent. They were paid for both donations at the end of the sport condition.

Immediately before their academic examination and during the first break in the control condition, the subjects had to describe their emotions on two questionnaires. First, they rated the intensity of six basic emotions (each on a nine-point-scale, ranging from 0 to 8). Figure 1 demonstrates that the students experienced significantly more anxiety [$t(11) = 11.1$, $P < 0.001$] and less pleasure [$t(11) = 5.6$, $P < 0.001$] during the first condition than during the second one. These mean differences reflect the observation that all subjects, without exception, experienced more anxiety and less pleasantness in the anxiety condition. Feelings of anger, disgust, sadness and surprise were not significantly different between both situations. Secondly, they described their emotions on the three dimensions of valence (happiness versus sadness), arousal (aroused versus calm) and dominance (dominant versus submissive) on language-free, graphic nine-point scales (Self-Assessment-Manikin, SAM; Bradley and Lang, 1994). When waiting for their examination, they felt less happy [$t(11) = 3.6$, $P < 0.01$], more aroused [$t(11) = 7.0$, $P < 0.001$] and less dominant [$t(11) = 3.7$, $P < 0.01$] than during the ergometer exercise.

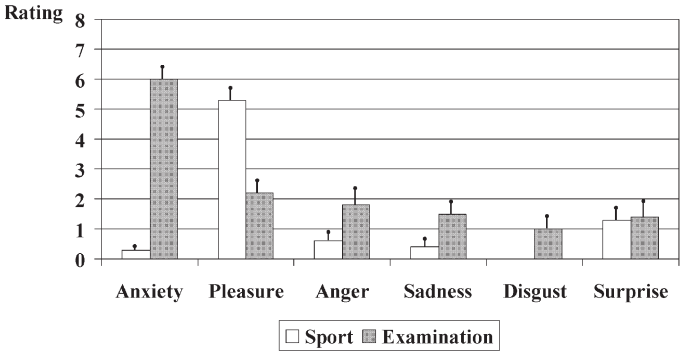

Figure 1 Emotions of sweat donors during the sport condition (bicycle riding) and while waiting on their academic examination (anxiety condition). Means and standard error of mean across all donors ($n = 12$). Feelings of anxiety were significantly stronger and feelings of pleasure were significantly weaker in the anxiety condition ($P < 0.001$, each).

The cotton pads were stored at –20°C. After all samples had been collected, they were homogenized and pooled according to the emotional condition. For the priming session, the pooled samples were placed into glass chambers (mean = 1.07 g cotton pad in each chamber, SD = 8 mg) of a constant flow (2*3 channel-) olfactometer (Burghart Co., Wedel, Germany; see Kobal, 2003). Chemosensory stimuli were presented birhinally by independent airstreams (100 ml/s) for 920 ms each with an interstimulus interval of 13 s. The temperature of the air flow at the exit of the olfactometer was 37°C and the humidity >80%. White noise of ~75 dB (A) was presented binaurally in order to prevent the participants from hearing the switching valves. A visual sign (white cross) was presented 1.26 s before activation of the odorous airflow and informed the subjects to start to inhale. The breathing procedure was practised before the test commenced and was easily learnt by all subjects.

Emotional priming

Pictures of faces (24.5 × 27.0 cm) were presented on a computer screen (19 in.) at eye level at a distance of ~0.95 m. All images are part of the Karolinska Directed Emotional Faces System (KDEF; Lundqvist *et al.*, 1998) and represent standardized facial expressions of emotions, presented by amateur actors.

The procedure was designed according to Höschel and Irle (2001): Task items consisted of the presentation of the prime (11.7 ms) followed by the presentation of a patterned mask (11.7 ms) and a target (47.0 ms). The prime–mask–target sequence was presented four times, with a free interval of 50 ms (Figure 2). Thereafter, subjects were instructed to press either the left button of a computer mouse if they believed they had seen a pleasant facial expression or the right button if they believed they had seen an unpleasant facial expression. The choice had to be made within 2 s and was accompanied by a visual presentation of a plus (to the

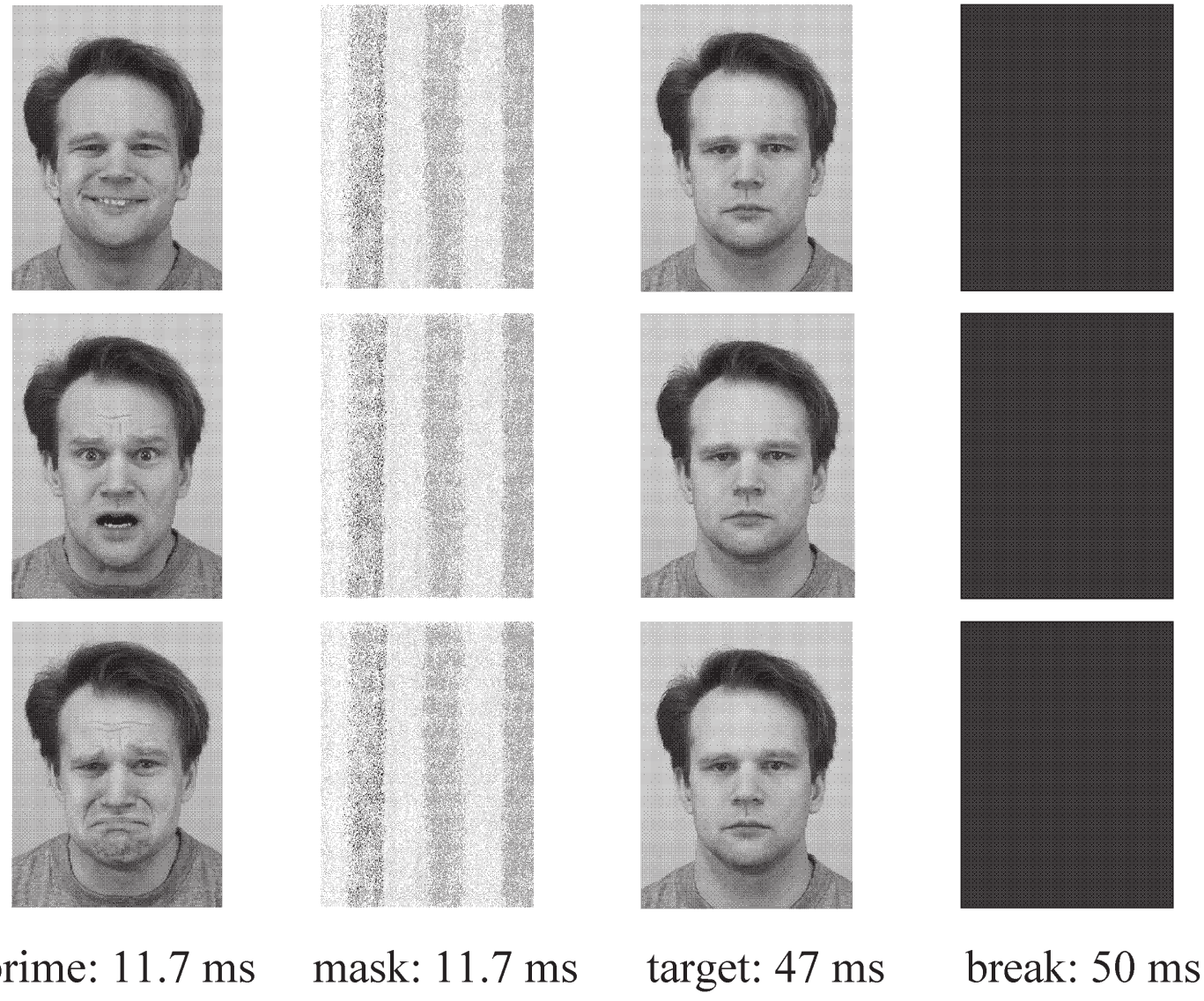

Figure 2 Prime–mask–target sequence. Within one trial, the sequence was repeated four times. The sequences were separated by the presentation of an empty black screen. Whereas the target always presented a neutral facial expression, the primes presented a happy, a fearful or a sad face.

left) and a minus (to the right) sign on the screen. After this, the subject's decision was displayed on the monitor (duration = 1 s). A break of 8.6 s (black screen) was included and 1.7 s before the next sequence started a white cross was presented in order to indicate the next picture presentation and to initiate inhaling. The odour was presented 1.26 s after the appearance of the cross and 0.44 s before the image sequence started and lasted until the sequence ended. The subjects were instructed to ignore the odours and to attend to the valence of the facial expressions.

Whereas the patterned mask and the target were always the same in each item, the primes varied between conditions. Images of a light-brown short-haired man served as primes and as target (Figure 2; according to a pre-test in our laboratory with 23 subjects, these images revealed best priming results.). A male face was chosen in order to be compatible to the male sweat signals. The target presented a neutral facial expression (KDEF No. AM17NES) and the primes presented a happy (KDEF No. AM17HAS), a sad (KDEF No. BM17SAS), or a fearful face (KDEF No. AM17AFS). Perceptional control items were interspersed among task items in order to control for a possible conscious perception of the primes. These control items consisted of images of a person who was clearly different from the light-brown haired male (dark- and long-haired; KDEF Nos AM01HAS, AM01SAS, AM01AFS). At the end of the experiment the subjects were asked whether they had seen a light-brown haired man only, or a light-brown haired man and additional persons, or different persons but no light-brown haired man. All but one subject stated that they had

seen the light-brown haired man only, one male claimed to have also seen other people and was excluded from all further analyses (see Participants section).

Design and procedure

At the beginning of the session, normal visual acuity was assessed using a standard line test (Oculus Co., Germany). Visus was a least 83% in all subjects. Thereafter, the subjects' ability to olfactorily detect the chemosensory samples was measured using a brief screening procedure. The participants had to discriminate the body odour from room air (three alternative task; test and control air were presented for 1 s each by the olfactometer, the presentation conditions were the same as in the priming experiment). Two trials were carried out for each chemosensory condition. Participants who failed to detect one of the body odours once were defined as non-detectors.

Afterwards, the participants were asked to subjectively judge the intensity, pleasantness, unpleasantness and familiarity of the chemosensory stimuli. Furthermore, they had to indicate whether the stimuli changed their emotional state. The quality and intensity of the participants' emotions were assessed with the same inventories used for the odour donors (SAM scale, basic emotions questionnaire).

The priming procedure started with a practise session, introducing the picture sequences and odour administration. This practise session comprised six trials. By this protocol, it was assured that the subjects understood the task correctly and adjusted their inhaling phases as instructed.

For the main priming session, 30 trials were undertaken (see Table 1). Each prime (facial expression of anxiety, sadness, happiness) was associated with each chemosensory condition (anxiety or sport) four times. During six trials the perceptional control primes were presented (each of the three facial expressions twice in conjunction with each odour condition). The whole priming procedure lasted ~7 min. At the end of the session the subjects rated the degree of their social anxiety on a questionnaire (U-Fragebogen; Ullrich and Ullrich, 1977).

Table 1 Study design

Trial no.	Perceptual condition	Prime condition	Chemosensory condition
1	Test condition	Fear	Anxiety
2	Test condition	Happiness	Anxiety
3	Test condition	Sadness	Sport control
4	Perceptual control	Happiness	Anxiety
5	Test condition	Fear	Sport control
6	Test condition	Sadness	Anxiety
7	Perceptual control	Sadness	Sport control
8	Test condition	Happiness	Sport control
9	Test condition	Fear	Anxiety
10	Test condition	Sadness	Sport control
11	Perceptual control	Fear	Sport control
12	Test condition	Sadness	Anxiety
13	Test condition	Happiness	Anxiety
14	Test condition	Fear	Sport control
15	Test condition	Happiness	Sport control
16	Test condition	Happiness	Anxiety
17	Test condition	Fear	Sport control
18	Perceptual control	Sadness	Anxiety
19	Test condition	Sadness	Sport control
20	Test condition	Happiness	Sport control
21	Test condition	Happiness	Anxiety
22	Test condition	Sadness	Anxiety
23	Perceptual control	Happiness	Sport control
24	Test condition	Fear	Anxiety
25	Test condition	Fear	Anxiety
26	Test condition	Sadness	Sport control
27	Test condition	Fear	Sport control
28	Perceptual control	Fear	Anxiety
29	Test condition	Happiness	Sport control
30	Test condition	Sadness	Anxiety

Statistical analyses

It was hypothesized that the chemosensory stimuli derived from sweat samples from the sport (control) condition do not alter the general priming effect of the facial expressions. Therefore, a one-way analysis of variance (ANOVA) was performed between the three priming conditions, and within the sports condition only. This test should reveal that positive primes (facial expression of happiness) evoke more positive judgements than negative primes (facial expressions of fear or sadness).

The main hypotheses focused on the effect of the chemosensory stimuli released by the sweat samples of the anxiety condition. However, so far it had not been possible to predict which priming condition will be affected by the anxiety-related samples. For example, it could be possible, that facial expressions of fear become more distinct in the context of the chemosensory anxiety signals or that, generally, facial expressions of negative affect become more distinct or that facial expressions of happiness become less distinct. Moreover, as the only study on sex-specific effects of chemosensory anxiety communication in humans, revealed gender differences, we had to carry out six independent tests, separated according to each sex and each priming condition. One-tailed *t*-tests (see Introduction) were carried out across the difference-values of positive judgements (%) between the sport and the anxiety condition (As

no trials were missing in either subject or condition, the transformation from the original data to percentage data was a simple linear transformation.) Due to the directed nature of our hypotheses, an overall analysis (e.g. by ANOVA) was not appropriate and the a priori *t*-tests were chosen (kurtosis and skewness analyses revealed that the difference-values of the positive judgements were normally distributed in each test). Additional analyses across the three priming conditions and within one odour condition only were avoided, because the effectiveness of putative chemosensory anxiety signals can only be interpreted with regard to the control condition. A Bonferoni correction of the α-error was calculated to prevent coincidental significance of the six parallel tests.

Results

Odour detection

Nine subjects (56.3%) could discriminate the sport odour from room air and 10 subjects (62.5%) the anxiety odour. Both detection rates did not differ significantly (McNemar test, $P > 0.20$). Six subjects (37.5%) were able to detect both odours. Due to this small number of subjects who had an olfactory impression of both chemosensory samples, it was abandoned to carry out statistical analyses of the body odour ratings.

Priming of facial affect perception in the context of chemosensory sweat signals

In the context of chemosensory signals of samples taken during exercise (control condition) the target faces were judged to be more positive when they were primed by the presentation of a happy face, than in the negative priming conditions with sad and fearful faces [$F(1,30) = 4.22$, $P = 0.02$, power (related to an α-error of 5%) = 0.70; see Figure 3]. Single comparisons revealed that the degree of different responses between the positive and the sad priming condition [$t(15) = 2.88$, $P = 0.01$] was similar to the degree of different responses between the positive and the fear priming condition [$t(15) = 2.54$, $P = 0.02$]. Targets primed with sad or fearful faces were judged to be equally negative [$t(15) = 0.43$, $P > 0.20$].

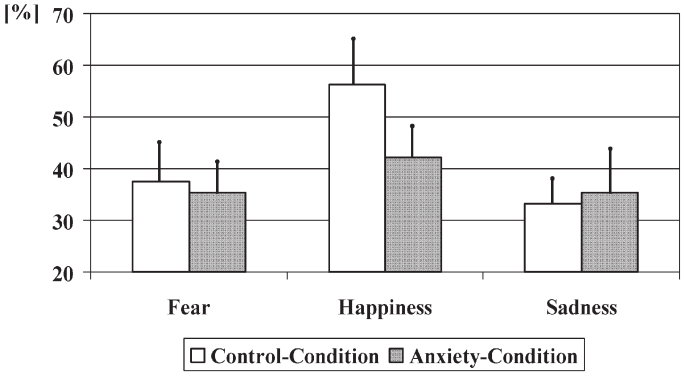

Figure 3 Percentages of positive judgements of the facial expressions in the chemosensory control and anxiety condition. Means and standard error of mean across all subjects ($n = 16$). In the control condition (chemosensory signals derived from sweat samples taken during sport), judgements of targets primed with happy faces were more positive than judgements of targets primed with sad or fearful faces ($P = 0.02$).

In the context of chemosensory signals of samples taken during a state of high anxiety, females judged targets primed with happy faces significantly less positive than in the control (sport) condition [$t(7) = 3.74$, P (α, corr.) = 0.02, power (related to an α-error of 5%) = 0.95; see Table 2]. However, the chemosensory stimuli from the anxiety sweat did not change the negative judgements of targets primed with sad or fearful faces. Face perception in males did not change with the condition of sweat collection.

Discussion

The present study could demonstrate that in females, positive priming of face perception is diminished in the context of chemosignals derived from sweat samples taken during a high state of anxiety. Basically, one could interpret the effect in the opposite direction and argue that the happy prime generally only works in the context of chemical signals of sweat sampled during a sport condition, whereas the chemosensory anxiety signals might have no effect. However, this argument is highly unlikely for two reasons: First, the visual priming paradigm has been established in a number of laboratories and yields robust priming effects of facial expressions (Murphy and Zajonc, 1993; Höschel and Irle, 2001; Öhman, 2004). Moreover, we used pictures from a standardized system (Lundqvist *et al.*, 1998) and our pre-tests confirmed the effectiveness of the primes. Second, to our knowledge there are neither animal data nor theoretical assumptions on the communicative function of chemicals derived from exercise sweat. On the other hand, the communication of anxiety (stress) has been well documented in many vertebrate species (see Wyatt, 2003). Summarizing, all empirical and theoretical evidence we are aware of, supports

Table 2 Percentage of positive ratings in the chemosensory control condition in relation to the percentage of positive ratings in the chemosensory anxiety condition (difference values of means: control-condition–anxiety-condition)

Sex of subjects	Emotional prime	Mean (±SD)	*t*-value (df = 7)	*P* (α, Bonferoni corrected)
Females	Fear	0.0 (48.2)	0.00	>0.20
	Happiness	25.0 (18.9)	3.74	=0.021
	Sadness	15.6 (32.6)	1.36	>0.20
Males	Fear	4.2 (33.0)	0.36	>0.20
	Happiness	3.1 (28.1)	0.31	>0.20
	Sadness	–19.8 (31.8)	–1.76	>0.20

the conclusion, that the reported effect is due to the effectiveness of chemosensory anxiety signals.

Chemosensory anxiety signals do not change the perception of negative facial expressions (sadness, fear). Thus, if negative emotional information is transmitted simultaneously via chemosensory and visual cues, chemosensory signals of anxiety do not seem to deliver additional salient information. It seems most probable that the percentage of negative responses reached a ceiling-level, which could not be changed in the context of additional negative social chemosignals.

However, if the two sensory channels deliver incompatible information (happiness and anxiety) the anxiety-related information seems to dominate stimulus processing. In general, it could be possible that either chemosensory signals are behaviourally more relevant in perceptual conditions delivering contrary multimodal social information, or that information related to a threat has a processing advantage compared to neutral or positive information. Chemosensory olfactory information is transmitted via the olfactory bulb directly to the medial and cortical amygdala (see Carmichael *et al.*, 1994; Otto *et al.*, 2000). Brain imaging studies revealed that the human amygdala is activated during odour perception (Savic *et al.*, 2000; Poellinger *et al.*, 2001), especially if the odours have a negative emotional valence (Zald and Pardo, 1997), or if they are emotionally salient and intense (Anderson *et al.*, 2003). Studies investigating the role of the amygdala in behavioural control showed that it is responsible for implicit immediate behavioural adaptations in emotionally meaningful situations (LeDoux, 2002). Therefore, the intrinsic connection between the olfactory bulb and the amygdala could explain the processing advantage of chemosensory olfactory information in brain structures responsible for the automatic initiation of behavioural responses (see Pause *et al.*, 2003). However, there is evidence that the human amygdala also processes auditory and visual social fear signals (Adolphs *et al.*, 1994; Morris *et al.*, 1996; Scott *et al.*, 1997). Even subliminal presentations of facial expressions of fear result in amygdala activations (Whalen *et al.*, 1998). Thus, fear signals of either modality could have an processing advantage, because they may initiate reflexive motor and physiological responses via the amygdala, independent of neocortical control (Armony and LeDoux, 2000).

The effectiveness of chemosensory anxiety signals reversing the priming effect of happy faces could be demonstrated exclusively in females. This result is in accordance with the study from Chen and Haviland-Jones (2000), who concluded that females can identify the emotional state of body odour donors better than males (however, in contrast to Chen and Haviland-Jones, we presented chemosensory substances from male donors only and can not rule out the possibility that males respond to emotional chemosignals collected from females). In line with the results of the present study, some studies indicate that odour perception in females is better than male odour perception (e.g. Yousem *et al.*, 1999). Moreover, it has recently been reported that the processing of visual emotional information activates different brain areas in males and females and that these differences are most prominent for negatively valenced stimuli (Lee *et al.*, 2002; Wrase *et al.*, 2003). As females responded with stronger brain activations to sad faces and emotionally negative pictures, Wrase *et al.* (2003) concluded that females may be more sensitive to aversive material in general. Thus, the observation that chemosensory anxiety signals affected females, but not males, could be due to a higher olfactory sensitivity or to a higher sensitivity to negative emotional situations in females.

The sweat donors participating in the present study described themselves as highly anxious, while waiting for their examination (anxiety condition). This contrasts with the medium level of anxiety of the subjects viewing anxiety-evoking movies (Chen and Haviland-Jones, 2000; Ackerl *et al.*, 2002). Moreover, the self-ratings suggest that all other basic feelings (pleasure, anger, sadness, disgust, surprise) were experienced at a very low level. Such an emotion-specific state during sweat sampling was not assessed by Chen and Haviland-Jones (2000) or by Ackerl *et al.* (2002). However, the donors also described themselves as being less dominant, and more aroused in the anxiety condition. Low dominance and high arousal might be intrinsically connected to ecologically valid anxiety conditions. An effect of arousal on the intensity of the sweat odours is unlikely because the olfactory detection rate for the pooled sweat samples was similar for both conditions. Moreover, the presentation of the chemosensory anxiety signals did not generally change the response behaviour to the primed facial expressions, but the effect was specific for the reversal of positive priming. This emotion-specific result strongly supports the conclusion that the chemosensory signals delivered anxiety related rather than arousal related information. However, in further studies, the arousal of the odour donors between different emotional conditions should be comparable. In our study, the lower arousal in the sport condition could be due to the fact that the ratings were taken during the first break (after 10 min of exercising) and not at the end of the sport condition (after 30 min of exercising).

So far, the mechanisms responsible for the production of emotional chemosignals remain speculative. Whereas traditionally it has been considered that emotional sweat is produced by apocrine glands (Boucsein, 1992), recent studies indicate that apoeccrine glands also contribute to the hedonic profile of body odour (Heckmann *et al.*, 2003). The production of emotional sweat in humans seems to be controlled by limbic brain areas, especially the amygdala (Asahina *et al.*, 2003). Furthermore, studies in rodents suggest that the release of chemosensory anxiety signals is related to the activity of the hypothalamus–pituitary–adrenal system (Mackay-Sim and Laing, 1981; Abel, 1994).

Even though the mediating systems in the production and perception of emotional chemosignals are not yet fully understood, we conclude that the results reported here strongly indicate that anxiety in humans can be chemosensorily communicated. Like in many other taxa of social animals, the release of alarm pheromones seems to be evolutionarily advantageous. The induction of a fright response (alertness associated with defensive action) in vertebrates (von Frisch, 1941) and mammals (Carr *et al.*, 1970; Mackay-Sim and Laing, 1981; Zalaquett and Thiessen, 1991) is obviously beneficial for the receiver as well as for the species, in socially living animals. As the benefit for the signaller is less obvious, the evolution of the specific response might be secondary to substances which are general products of fighting of injured animals (Wyatt, 2003).

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