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VISUAL ATTENTION TO FACES IN INTERSEXUAL AND INTRASEXUAL SELECTION

VIZUÁLNÍ POZORNOST VĚNOVANÁ OBLIČEJŮM V MEZIPOHLAVNÍM A VNITROPOHLAVNÍM VÝBĚRU

Doctoral thesis

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ABSTRACT

This thesis focuses on visual attention towards faces in intersexual and intrasexual selection. It consists of two main parts. The first part begins with a brief overview of the intersexual and intrasexual selection mechanisms. Within the framework of intersexual selection, facial attractiveness is discussed, with its putative link to health and immune system function. Within the framework of intrasexual selection, the thesis focuses on perceived facial dominance and formidability and their connection to actual characteristics. Moreover, specific facial traits and morphological features connected with judgements of attractiveness, dominance, and formidability are described. Given the scarce evidence of direct visual attention towards facial features connected with judgements of facial attractiveness and dominance or formidability, we discuss the eye-tracking method, which can measure direct visual attention. A brief summary of eye-tracking studies focusing on visual attention to faces, especially during attractiveness and dominance assessments, is provided. Finally, the thesis focuses on how individual sensory modalities (visual, olfactory and vocal) relate to each other in the perception of individuals and the detection of cues to an individual's quality.

The second part of this thesis includes five empirical studies. The first study shows that perceived facial attractiveness, healthiness, skin healthiness and facial skin colouration provide limited cues to immunoreactivity and facial skin colouration is only connected to perceived characteristics. The evidence that facial attractiveness cues an individual's immunocompetence remains equivocal, but it seems that individuals are generally sensitive to more immediate changes in appearance caused by current illness. In the second study, we observed that the individuals' faces, following immune system activation by vaccination, were perceived as less attractive and healthy. Though facial appearance and specific facial features are thought to cue numerous individual's qualities, direct visual attention is not often investigated. The third paper is an eye-tracking study focusing on visual attention to faces in intersexual and intrasexual selection. It showed that women gave more visual attention to the faces of potential partners than to rivals, but they also gave more visual attention to both potential partners and rivals than men did. Variations in visual attention with respect to the rater's sex and rating context for facial features proposed as important in respective judgements, such as cheeks and chin, were detected. Nonetheless, the eyes, nose and mouth received most of the visual attention. The fourth study focused on visual attention towards male faces and features under judgements of attractiveness and formidability, considering the target's level of attractiveness and formidability. Faces with a medium level of formidability received more visual attention than those with a high level of formidability, but no association between the target's level of attractiveness and visual attention was found. Similar to the third study, the eyes, nose, and mouth captured the most visual attention. Variations were observed in visual attention in relation to the rater's sex or target's level of attractiveness and formidability towards other facial features, such as the chin. The fifth study shows a weak positive association between body odour and facial attractiveness, as well as body odour and vocal attractiveness. Given the strength of this association, it appears that faces, body odour, and voices provide non-redundant information about an individual's mating quality.

KEYWORDS

Mate choice; competition; face perception; attractiveness; dominance; formidability

ABSTRAKT

Předkládaná disertační práce se věnuje vizuální pozornosti vůči obličejům v mezipohlavním a vnitropohlavním výběru. Skládá se ze dvou hlavních částí. První část nejprve poskytuje stručný přehled mechanismů mezipohlavního a vnitropohlavního výběru. V rámci mezipohlavního výběru je diskutována atraktivita obličeje a její možná souvislost se zdravím a funkcí imunitního systému. V rámci vnitropohlavního výběru se práce zaměřuje na vnímanou obličejovou dominanci a schopnost obstát ve fyzické konfrontaci a jejich souvislost se skutečnou dominancí a schopností obstát ve fyzické konfrontaci. Dále jsou popsány konkrétní obličejové rysy spojené s vnímanou obličejovou atraktivitou, dominancí a schopností obstát ve fyzické konfrontaci. Vzhledem k nedostatečné evidenci ohledně přímé vizuální pozornosti vůči obličejovým znakům spojeným s hodnocením obličejové atraktivity či dominance a schopností obstát ve fyzické konfrontaci je diskutováno využití metod eye-trackingu, které přímé sledování vizuální pozornosti umožňuje. Následuje stručné shrnutí eye-trackingových studií, které se věnují vizuální pozornosti vůči obličejům, zvláště při hodnocení atraktivity a dominance. Nakonec se práce zaměřuje na to, jak spolu souvisí jednotlivé smyslové modality (vizuální, olfaktorická a akustická) při percepci jedinců a rozpoznávání vodítek kvality.

Druhá část této disertační práce zahrnuje pět empirických studií. První studie ukazuje, že vnímaná atraktivita obličeje, zdraví, zdraví pokožky a barvy pokožky obličeje spíše neposkytují vodítka k imunoreaktivitě a že zbarvení pokožky je spojeno pouze s některými vnímanými charakteristikami. Důkazy pro to, že by obličejová atraktivita poskytovala vodítka k imunokompetenci jedince, zůstávají nejednoznačné, ale zdá se, že lidé jsou obecně vnímaví k bezprostřednějším změnám ve vzhledu způsobených aktuálním onemocněním. Ve druhé studii jsme zjistili, že obličeje jedinců s aktivovaným imunitním systémem byly vnímány jako méně atraktivní a zdravé. Ačkoli se předpokládá, že vzhled obličeje a konkrétní obličejové rysy slouží jako vodítka ke kvalitě jedince, přímá vizuální pozornost vůči nim není často zkoumána. Třetím článkem je eye-trackingová studie, která se zaměřuje na vizuální pozornost vůči obličejům v kontextu mezipohlavního a vnitropohlavního výběru. V této studii se ukázalo, že ženy věnovaly více vizuální pozornosti obličejům potenciálních partnerů než rivalek, ale také věnovaly více vizuální pozornosti než muži jak potenciálním partnerům, tak rivalkám. Ve vztahu k pohlaví hodnotitele a hodnotícímu kontextu jsme nalezli jsme rozdíly ve vizuální pozornosti vůči rysům obličeje, které byly předchozími morfologickými studiemi navrženy jako důležité pro příslušná hodnocení, např. tváře a bradu. Oči, nos a ústa však získaly většinu

vizuální pozornosti. Čtvrtá studie se zaměřila na vizuální pozornost vůči mužským obličejům a jejich rysům při hodnocení atraktivity a schopnosti obstát ve fyzické konfrontaci a zároveň vzala v potaz míru přisouzené atraktivity a bojeschopnosti hodnoceného obličeje. Obličeje se střední mírou bojeschopnosti získaly více vizuální pozornosti než obličeje s vysokou mírou bojeschopnosti, ale nenalezli jsme žádnou souvislost mezi mírou atraktivity hodnoceného obličeje a vizuální pozorností. Nejvíce vizuální pozornosti přitahovaly oči, nos a ústa, podobně jako ve třetí studii. Byly pozorovány odchylky ve vizuální pozornosti v závislosti na pohlaví hodnotitele a míře atraktivity a bojeschopnosti hodnoceného obličeje vůči dalším obličejovým rysům, např. bradě. Pátá studie ukazuje slabý pozitivní vztah mezi tělesnou vůní a atraktivitou obličeje a mezi tělesnou vůní a atraktivitou hlasu. Vzhledem k síle tohoto vztahu se zdá, že obličeje, tělesná vůně a hlasy poskytují neredundantní informace o kvalitě jedince.

KLÍČOVÁ SLOVA

Výběr partnera; kompetice; percepce obličeje; atraktivita; dominance; schopnost obstát ve fyzické konfrontaci

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PART I.

INTRODUCTION

Physical appearance is shown to be incredibly important in everyday social interactions, and it has been demonstrated that people are especially attentive towards faces (Gillath et al., 2017; Hewig et al., 2008). In as little as 100 ms, individuals can form impressions of others from their faces and make judgements about their likeability, trustworthiness, competence, aggressiveness, or attractiveness (Willis & Todorov, 2006).

Attractiveness can have a vast impact on an individual's life, as it has been found to positively correlate with judgements of many socially desirable traits, such as being intelligent, emotionally stable, responsible, or trustworthy (Batres & Shiramizu, 2022). An individual's perceived attractiveness can influence them from early school age, where more attractive children were shown to get higher scores in achievement tests (Salvia et al., 1977), to when applying for a job later in life, as more attractive individuals are generally preferred (Dipboye et al., 1975). Even during criminal trials, more attractive defendants were found to receive less severe sentences (Stewart, 1980). Another highly important area of an individual's life, which is largely influenced by physical appearance and facial attractiveness, is mate choice (Buss, 2015).

There are undoubtedly some individual and cross-cultural differences (Fiala et al., 2021), but there seems to be a high level of agreement on which faces (or facial traits) are attractive (Perrett et al., 1994; Rhodes, 2006). In fact, even babies were shown to prefer and look more at physically attractive adult faces (Griffey & Little, 2014; Langlois et al., 1991; Slater et al., 1998). Evolutionary research suggests that the perception of certain facial traits as attractive is not arbitrary. Rather, these attractive facial traits were proposed to cue numerous aspects of individuals' quality, for example, their actual health or the function of their immune system (Little, 2014; Rhodes, 2006; Thornhill & Gangestad, 1999), which might be important qualities in a mating partner as they can lead to higher reproductive success.

Moreover, evolutionary sciences emphasise that in mate choice, there is another important factor besides the actual choice of partner, and that is the competition with potential rivals, who might disrupt the access to potential partners (Puts, 2010). Assessment of rivals based on physical appearance could influence the nature and outcome of the antagonist interactions. Adequate assessment can aid in minimising the losses (injuries, loss of resources) and maximising the gains (access to resources and partners) (Chen Zeng et al., 2022). In fact, people were shown to be able to judge formidability cues from facial appearance (Třebický et al., 2013, 2013), and specific facial traits and morphological features were likewise connected with perceived but also actual formidability and dominance (Little et al., 2015; Windhager et al., 2011).

This thesis focuses on visual attention to faces in intersexual and intrasexual selection. The first part consists of an overview that introduces and describes the context of empirical studies that are presented in the second part of this thesis. In the first part, I will shortly introduce the studies and describe their main points or contributions to the field, but their objectives are described in depth in respective papers.

The first part is divided into five chapters. **Chapter 1** introduces intersexual and intrasexual selection and the interplay between them. **Chapter 2** is dedicated to facial attractiveness and its possible connection to the underlying qualities of an individual, while it especially focuses on the immune system and current health. Further, the facial traits and morphological features proposed as important for attractiveness judgements are introduced. **Chapter 3** is devoted to facial dominance. Firstly, we discuss the concept of dominance, as well as its connection to fighting ability and formidability or masculinity. Then, we present evidence for the links between facial characteristics and actual dominance and formidability. Lastly, similar to facial attractiveness, we discuss the specific facial traits and morphological features identified as important in judgements of facial dominance and formidability. It is gradually shown that numerous perception and geometrics morphometrics studies identify certain facial features as important for judgements of attractiveness and dominance, but direct visual attention to them, which would further highlight their importance, hasn't been extensively examined. In **Chapter 4,** we discuss the use of the eye-tracking method and the most relevant eye-tracking studies in this area of research. Moreover, we show whether there are context and sex-dependent differences in visual attention to faces of potential partners versus rivals and whether faces with varying degrees of facial attractiveness and formidability capture visual attention differently. In **Chapter 5**, we take a closer look at how the modalities (visual, acoustic and olfactory) work together in the perception of individuals. We discuss two main hypotheses: the "back up" signals hypothesis, which proposes that each modality provides the same redundant information and the "multiple messages" hypothesis, which claims that each modality provides distinct and non-redundant information about the conspecific. The second part starts with **Chapter 6,** which focuses on the association between immunoreactivity and perceived facial attractiveness, healthiness, skin patch healthiness and facial colouration. **Chapter 7** investigates changes in perceived facial attractiveness and healthiness, facial skin colouration, body odour and vocal attractiveness after activation of the immune system by vaccination. In **Chapter 8,** visual attention to the faces of potential partners and rivals is explored. **Chapter 9** focuses on visual attention to male faces during judgements of attractiveness and formidability. It also considers the target's level of attractiveness and formidability and whether they have any effect on visual attention. **Chapter 10** presents a meta-analysis and systematic review aiming to shed light on the association between individual modalities (olfactory and visual and olfactory and vocal) in attractiveness assessments.

1 INTERSEXUAL AND INTRASEXUAL SELECTION

Sexual selection was a proposed mode of natural selection that allowed the explanation of traits that don't necessarily aid the organism's survival or can potentially even lower the survival chances, e.g., peacock's plumage. However, they can substantially increase an organism's chance to reproduce (Buss, 2015; Darwin, 1871). The two mechanisms of sexual selection are intersexual selection, in which individuals of one sex choose individuals of the other possessing certain development of traits as mates, and intrasexual selection, in which individuals compete with other individuals of their own sex for access to mates. Due to a pertaining consensus of higher investment into reproduction on the side of women, they are believed to be, and usually are, the choosier sex, while men are believed to be more competitive for mates (Barber, 1995; Buss, 2015; Puts, 2010). These two mechanisms of sexual selection are believed to drive the specific development of facial and body morphology (Puts, 2010; Třebický & Havlíček, 2021).

Intersexual selection likely exerts selection pressures which shape the development of heritable traits that are considered attractive. These attractive traits are, in turn, believed to serve as cues to various aspects of an individual's mating quality, including health, immunocompetence, or developmental stability, to name a few (Stephen & Luoto, 2023). Preference for such traits is proposed to be adaptive and likewise heritable, as their presence in a mating partner can lead to higher reproductive success through direct benefits, such as parental care, access to resources and protection, and indirect benefits, which may include good genetic material for an offspring (Kirkpatrick & Ryan, 1991; Thornhill & Gangestad, 2006). Especially in species with higher male parental care, it is not the case that mating partners would possess just direct or only indirect benefits, but they often possess a combination of both on a continuum (Qvarnström & Forsgren, 1998).

Intrasexual selection likewise poses selective pressures, influencing the development of traits connected to success in competition and confrontation, which are linked to perceived and actual dominance, formidability, masculinity and fighting ability (Fink et al., 2007; Stephen & Luoto, 2023; Třebický et al., 2012). Cues to dominance and formidability and their assessment could be pivotal in determining who to recruit as an ally and when to engage in a confrontation with a rival or opt for withdrawal instead (Sell et al., 2012; Třebický & Havlíček, 2021). As a result of these choices, individuals can attain the benefits of winning, such as gaining access to mates and resources, while also avoiding substantial costs of losing, including injury and even death (Puts, 2010).

Though the mechanisms of sexual selection may seem relatively straightforward, the interplay between them and how they shaped the development of specific traits is far from that. The available evidence largely highlights the importance of mate choice in the development of morphological traits (Dixson et al., 2007; Saxton et al., 2016). However, other studies proposed that in the case of male morphological traits, intrasexual selection may have played a very important role, and some suggest it as the primary mechanism of sexual selection in males (Kordsmeyer et al., 2018; Puts, 2010). Based on the available evidence, we can assume that there wasn't a single mechanism which would solely contribute to specific trait development. Rather, they influenced the trait development in conjunction and interactively. This can be further attested by studies focusing on perception and preferences.

We can first focus on whether the two mechanisms of sexual selection go hand in hand. In such case, we would expect that women would prefer formidable men, as formidability could be a cue to mate quality and choosing a formidable partner could provide both direct and indirect benefits, i.e., access to resources and protection of offspring or heritable traits of formidability (Třebický et al., 2012). Studies in this area of research usually don't directly focus on formidability but rather on related concepts such as masculinity or dominance. These concepts are also sometimes used interchangeably in studies, see Chapter 3. Investigations focusing on facial masculinity show that the evidence regarding women's preference for this trait in men is somewhat mixed (Burriss et al., 2014; DeBruine et al., 2010; Rhodes et al., 2003). Hill et al. (2013) have shown that women preferred more attractive but not more masculine male faces and voices, and Třebický et al. (2012) concluded that masculine traits are perceived as attractive, but their higher development increases perceived dominance more than perceived attractiveness. This may be the case because studies show that the choice of a formidable partner may not carry the benefits of better parental care or access to resources for women, and even if they do, it may pose significant disadvantages, as formidable men may direct aggression towards their partners (Qvarnström & Forsgren, 1998; Snyder et al., 2008).

Therefore, women likely calibrate their preferences based on the trade-off between the cost and benefits of choosing a formidable partner, which might aid in explaining the ambiguous results of studies investigating female preference for male formidability traits (Qvarnström & Forsgren, 1998). Moreover, Třebický et al. (2012) have also stressed that the preference for specific traits and their development might not follow a linear relationship, which is still sometimes anticipated in the current studies, and rather correspond to a more curvilinear pattern, such as perceived attractiveness would increase up to a certain point of a trait development and above the threshold, their attractiveness would decrease. Frederick & Haselton (2007) suggested that very high levels of masculinity might be deemed unattractive since those men could be perceived as threatening by women. Consequently, some of the masculine traits have been proposed to evolve rather for male intrasexual competition than mate choice (Hill et al., 2013).

Though intrasexual selection is a researched force for the development of certain traits in men, in women, the area is not receiving such attention. More recent research mentions that women may indeed compete for higher quality mates, that is, for those that can provide, e.g., superior parental care, resources, etc. (Campbell, 2015; Fisher et al., 2013; Puts, 2010; Rosvall, 2011). Previous literature has shown that there is a higher prevalence of physical aggression (one of the ways to establish a dominant position and/or acquire the benefits from the conflict) in conflicts among men than in women (Archer, 2004; Knight et al., 1996, 2002), even though both sexes seem to experience anger comparably (Archer, 2004). This could point towards the fact that women might be more calibrated for avoiding the cost of overt physical aggression, possibly due to the consequences the mother's injuries or death may bring to the offspring (Campbell, 2015). Authors, therefore, seem to agree that female intrasexual competition generally takes subtler forms, such as own attractiveness enhancement (Puts, 2010) and self-promotion (wearing make-up or certain clothes) or indirect forms, such as derogation, which includes making a rival less attractive to the members of the opposite sex through gossip (attacking the target's fidelity or appearance) or social exclusion (Campbell, 2015; Fisher et al., 2013; Vaillancourt, 2013). On the other hand, the situation becomes quite different under specific ecological and cultural conditions, such as when the sex ratio becomes more female-biased or when there is a high variation in male resources, and the stakes in securing the one who possesses them will be high, for example, in deprived areas (Campbell, 2015). In such cases, it has been documented that women engage in overt physical aggression (Ness, 2004; Rosvall, 2011).

Rosvall (2011) concludes that (behavioural) traits leading to female-to-female competition aren't merely nonadaptive by-products of selection on males. Instead, sexual selection seems to favour female-to-female competition. Therefore, we may also ask whether certain physical traits refer to women's dominance, analogous to male formidability (as most studies don't focus specifically on female formidability; for more on this, refer to Chapter 3), and what their connection to attractive traits is. Some studies provided evidence that women compete with each other in terms of attractiveness (Fink et al., 2014; Fisher, 2004). Drawing a parallel to male-to-male competition, this might suggest that attractive traits could also qualitatively correspond to female dominance, at least to some extent. However, this is usually not the case (for a positive relationship between perceived facial attractiveness and dominance, see Gallup et al., 2010; Gonzalez-Santoyo et al., 2015). It was shown that one of the factors that raise female facial dominance is maturity, but more mature (i.e., categorised as more dominant in that study) female faces are not perceived as attractive (Keating, 1985) and generally higher age in women lower their attractiveness, as demonstrated by several studies (for review, see Little, 2014; Thornhill & Gangestad, 1999). Further, more masculine facial features in women were perceived as more dominant (Quist et al., 2011; Watkins et al., 2012). However, studies steadily show that it is facial femininity that is considered attractive in women, not facial masculinity (Cunningham, 1986; Perrett et al., 1998; Rhodes, 2006; Van Dongen, 2014), and it should be noted that the relationships between youth and facial femininity/masculinity are interconnected, as the female face is masculinised with age (Fitousi, 2021; Thornhill & Møller, 1997). Moreover, Fink et al. (2014) showed that women with more feminine faces were rated the highest on the perceived intrasexual competition. Further, a study showed a significant positive correlation between perceived facial attractiveness and assertiveness, a behavioural characteristic often associated with dominance (Cunningham, 1986).

The interplay between intersexual and intrasexual selection on the development of traits remains still unresolved. For men, the studies indicate some overlap between attractive and dominant or formidable facial traits (DeBruine et al., 2010), though more nuanced, curvilinear relationships between the preference for certain traits and their development have been suggested (Frederick & Haselton, 2007). Moreover, based on the available evidence, there is little overlap, if any, between attractive and dominant facial traits in women (Cunningham, 1986; Van Dongen, 2014). Future studies should explore the connection between female facial attractiveness and (perceived and actual) dominance further, as there have been relatively few studies on this topic compared to males. Lastly, one can expect both sexes to be attentive to cues of attractiveness and formidability and be able to make respective assessments. For men, attractive traits in women might provide information about the quality of potential partners, while attractive and formidable traits in other men might provide cues towards the quality of potential rivals. For women, both attractive and formidable traits in men might provide cues primarily for the quality of potential mates, while being attentive towards attractive and dominant traits in women might provide relevant information for female intrasexual competition. Whether this is the case is explored throughout Chapters 2, 3, 6, 8 and 9.

2 FACIAL ATTRACTIVENESS

In the previous chapter, we referred to attractive traits, which are considered cues to an individual's mating quality, whose preference is expected to lead to direct and indirect benefits. Regarding faces, attractive traits can be either morphological (e.g., symmetry, averageness and sexual dimorphism or specific development of facial features) or non-morphological (e.g., facial skin colouration or its texture), and it has been believed both types can refer to several aspects of an individual's quality (for review, see Little, 2014; Little et al., 2011).

It has been largely believed that attractive traits correspond to the health of their carrier, although studies investigating this topic have delivered contradictory results (Hume & Montgomerie, 2001; Jones et al., 2021). One possible reason for these discrepancies could be the loose definition of the term health for the purpose of these studies. We believe that it is important to distinguish between the overall "general" health of the individual, e.g., their immune system quality and loosely developmental stability, that is, whether individuals underwent stable development under the influence of environmental pressures. Then, there is current health status, e.g., whether the individual is currently ill and thus someone to avoid to not to get infected. It's important to acknowledge that our current focus is specifically on exploring the link between immune system quality and current health and facial attractiveness. Nonetheless, facial attractiveness has also been proposed to cue other facets of an individual's quality (Roberts et al., 2005; Thornhill & Gangestad, 1993), which we don't further discuss due to the scope of this thesis.

2.1 Immune system quality

When focusing on an individual's immunity and its relation to facial attractiveness, studies gradually moved from using self-reports or medical history records of past and current health (about the presence or absence of illness) (Thornhill & Gangestad, 2006; Zebrowitz & Rhodes, 2004), to employing direct measures of the immune system (Cai et al., 2019; Pátková et al., 2022; Phalane et al., 2017), though the evidence didn't get much clearer.

There are several ways to assess immune system quality (for a summary of some of the methods studies used, see Pátková et al. 2022). One such direct measure can be the assessment of immunoreactivity, i.e., how (strongly) the immune system reacts to an antigen. Phalane et al. (2017) tested the immunoreactivity by measuring the immune response after injection of bacterial lipopolysaccharide (LPS), which elicits an immune reaction and a strong feeling of being unwell in participants. The study found a positive relationship between levels of cytokines, peptides stimulating immune response, and perceived attractiveness ratings in men. Another way to measure immune system reactivity is through vaccination. Studies have utilised greater antibody response after vaccination as an indication of better protection against infection (Burns & Gallagher, 2010). Rantala et al. (2012) indeed found a positive association between male facial attractiveness and higher levels of hepatitis B antibodies, though Skrinda et al. (2014) did not, and null results were also found for women (Rantala et al., 2013). Moreover, in our study, we found no relationship between the immune system reactivity, measured by an increase of specific antibodies after vaccination against hepatitis A, meningococcus, and perceived male facial attractiveness, healthiness and skin patch healthiness (which we employed to avoid possible confounding effects of face morphology) rated by women (Pátková et al., 2022). Therefore, it seems that the evidence for an association between perceived facial characteristics and individuals' immunoreactivity remains equivocal.

It has been proposed that one of the possible ways the immune system functions and physical condition could be manifested in the face is through facial colouration (Stephen et al., 2009, 2011). Studies in this area of research traditionally use CIE L*a*b* colour space, where L* stands for lightness (white-black axis), a* for redness (green-red axis) and b* for yellowness (blue-yellow axis). Skin yellowness is generally perceived as attractive and healthy (Phalane et al., 2017; Stephen et al., 2011) and is affected by carotenoids, natural pigments acquired from food. Carotenoids can cause disease resistance by destroying free radicals and reducing oxidative stress. A higher level of skin redness is perceived as attractive and healthy (Stephen et al., 2009). Skin redness is connected with blood perfusion and oxygenation, which are connected to physical fitness (Johnson, 1998) and cardiovascular health (Jonasson et al., 2022) and may thus correspond with an individual's condition. In women, facial lightness is generally perceived as attractive (Badarudozza, 2007; Kleisner et al., 2017), while for men, darker skin is rated as more attractive (Carrito et al., 2016). Moreover, higher melanin levels partly cause darker skin and melanin interaction with melatonin can possibly have an effect on the periodicity of the immune system as well as cytokine production (Guerrero & Reiter, 2002; Slominski et al., 2008). A relationship between higher facial skin yellowness and higher immune response has been found (Phalane et al., 2017), but other studies didn't corroborate this result (Foo et al., 2017a). To the best of our knowledge, the relationship between other facial colours than yellowness and immune function hasn't been inspected before.

In our study, we showed no statistically significant association between facial colouration (lightness, yellowness, redness) and antibody levels in men. When female raters were presented with full male facial photographs, higher forehead redness was perceived as less attractive and healthy, and cheek redness was perceived as less healthy when judged from skin patches (Pátková et al., 2022). One of the differences between the previous studies (Foo et al., 2017a; Phalane et al., 2017) and our study is that we employed facial colouration on the cheeks and forehead separately, as the colour measurements from cheeks and forehead in our study were only loosely associated. The previous studies created one mean value from several measurements from different parts of the face. It has been previously shown that variation in colour on different parts of the face has an effect on perceived characteristics (Jones et al., 2016), and our results support that (Pátková et al., 2022).

Jones et al. (2021) summarise the studies exploring the topic and conclude that there is little compelling empirical support for the hypothesis that facial attractiveness and individual facial characteristics, such as colouration, signal immune system functioning. They further suggest that future studies should focus on facial attractiveness in relation to a lifestyle that comes with health benefits, including a healthy diet (Jones et al., 2021). That facial attractiveness reflects a more immediate individual's condition is well possible. One such example could be found in the evidence that more oxygenated blood and higher skin perfusion are perceived as attractive (Stephen et al., 2009), which are in turn associated with cardiovascular and pulmonary health (Johnson, 1998; Jonasson et al., 2022; Myers, 2003), as noted at the outset. These could be, to some extent, among others, influenced by physical exercise (Myers, 2003), while generally preferred higher facial yellowness can be influenced by a diet rich in fruits and vegetables (Appleton et al., 2018).

2.2 Current health status

Another factor that influences the individual's appearance over rather short periods of time is a current illness. The studies agree that individuals are sensitive to the current health status of their conspecifics, which would be adaptive as it enables individuals to avoid those individuals (e.g., mating partners) that could transmit infectious diseases to them (Kirkpatrick & Ryan, 1991). This could be ascribed to the functioning of the behavioural immune system, which is believed to be comprised of a set of psychological mechanisms providing an extra line of defence, in addition to the physiological immune system, by avoiding possible sources of pathogens. For a review, see Schwambergová et al. (2020). While the behavioural immune system significantly influences our perspective here, we, of course, acknowledge the exceptions. For instance, individuals, like parents caring for their ill children or doctors attending to patients in hospitals, do not consistently avoid the ill, and the instances of such exceptions are numerous. In such cases, the physiological immune system is favoured (Ackerman et al., 2018).

Studies have shown that changes in current health status and immune system activation (i.e., the body's response to antigen) can be perceivable through changes in body odour (Olsson et al., 2014) or gait (Sundelin et al., 2015). Faces seem to be no exception and carry information about the current health status of the individuals as well. It has been demonstrated that individuals in facial photographs whose immune system was activated through the application of LPS were rated as less attractive, healthy and socially desirable (Regenbogen et al., 2017). Moreover, raters were found to identify ill participants (injected with LPS) from facial photographs at a level above chance (Axelsson et al., 2018). One of the tell-tale signs of the current illness might be having, e.g., lighter skin and hanging eyelids (Axelsson et al., 2018). That participants' faces are lighter and also less red after immune system activation by LPS was found in another study, which employed direct measures of facial skin colouration (Henderson et al., 2016), as opposed to Axelsson et al. (2018). The study attributed these changes to vasoconstriction, where the body tries to conserve heat during the initial stages of fever. In our study, we showed that men's facial photographs collected 14 days after vaccination against hepatitis A/B and meningococcus were rated by women as less attractive and healthy. However, we haven't detected any changes in facial colouration (lightness, redness, yellowness) before and after vaccination. Therefore, facial colouration likely didn't mediate the change in facial characteristics ratings. We have also detected an increase in perceived body odour attractiveness and no changes in perceived vocal attractiveness. To sum up, faces and body odour may reveal activation of the immune system (see Chapter 7). The results of the studies (Regenbogen et al., 2017; Schwambergová et al., under review) also suggest that the interplay between individual modalities might be important, and it will be discussed in Chapters 5, 7 and 10.

2.3 Attractive facial features

An extensive body of literature has been investigating which facial features are perceived as attractive. The studies generally concluded that facial symmetry is considered attractive (Perrett et al., 1999; Rhodes et al., 2001; Stephen et al., 2014), as well as averageness (that is, how much certain face looks like the majority of other faces in a population) (Little, 2014; Rhodes et al., 1999). However, we will not cover these in detail. Instead, we will concentrate on those that are more closely related to the main topics of this thesis, such as specific feature development and shape connected to judgements of attractiveness and dominance/formidability and sexual dimorphism.

Morphological studies, some of them using methods of geometric morphometrics (GMM), describe which specific facial features play a role in attractiveness judgements. In men, faces with wider mouths, fuller lips, more angular jaws, pointed or prominent chin, thicker eyebrows, prominent cheekbones, large eyes, small noses and vertical stretching in the regions of eyebrows, eyes and mid-face were shown to be perceived as attractive (Cunningham et al., 1990; Windhager et al., 2011). Women's faces that receive higher attractiveness ratings have fuller lips, smaller noses, less angular jaws and higher brows (Abend et al., 2015; Pflüger et al., 2012; Schaefer et al., 2006); small but pointed chin (Valenzano et al., 2006) and large eyes (Cunningham, 1986).

One of the factors influencing the perceived attractiveness of specific facial features might be the preference for sexual dimorphism in faces, that is, facial masculinity and femininity. (Komori et al., 2009; Rhodes, 2006). Women generally have smaller noses, fuller lips and cheeks, smaller jaws and less prominent supraorbital arc than men (Bannister et al., 2022). Concordantly, men have bigger noses, more prominent supraorbital arcs, wider jaw, more prominent cheekbones, longer chin, and smaller eyes (Fink et al., 2005; Gangestad & Thornhill, 2003; Komori et al., 2009). Therefore, how typically male-like or female-like the individual looks corresponds to their facial masculinity and femininity.

In women, facial femininity is generally preferred (Cunningham, 1986; Perrett et al., 1998; Rhodes et al., 2003), and we can see that the above-discussed attractive facial features largely overlap with feminine facial features. However, in men, the evidence for preference of facial masculinity is mixed (Rhodes, 2006), and some studies show that higher facial masculinity in men is, in fact, not perceived as more attractive (Burriss et al., 2014; Penton-Voak & Chen, 2004; Perrett et al., 1998; Rhodes et al., 2003). Also, based on the above-discussed morphological studies (Cunningham et al., 1990; Windhager et al., 2011), it seems that a combination of rather feminine and masculine facial features might be perceived as attractive in men. We could also assume a curvilinear relationship between facial masculinity and attractiveness, where moderate over extreme levels of masculinity would be preferred

(Rhodes, 2006). This is due to a certain threshold where extreme levels of masculinity could correspond to excessive dominance and formidability and unfavourable social characteristics in a partner, as opposed to advantageous qualities such as resource securing (e.g., Keating, 1985). Additionally, there also seems to be a high variation in women's individual preferences for male facial masculinity, which can be affected by numerous factors, including societal-level measures of development and pathogen load. For more details on this topic, see e.g., DeBruine et al. (2006, 2010), Marcinkowska et al. (2019), Moore et al. (2013). The results of the studies might also be affected by different methodologies studies use, as they range from utilisation of "natural" faces to employing computer-manipulated facial stimuli that might not reflect the natural variation of feature development in the population (Dong et al., 2023).

In this chapter, we have discussed the links between facial appearance and possible underlying qualities of individuals, namely health and its two components of immune system quality and current health status. We have shown that the evidence regarding immunocompetence and facial characteristics remains equivocal (Jones et al., 2021; Pátková et al., 2022). We should also point out that the immune system is an intricate cascade of processes with various components. Stimulating the immune system by vaccination, or LPS, and focusing on changes in antibodies might not provide a comprehensive understanding of its function and, therefore, be rather simplistic. Future studies might try to employ other measures of the immune system and acquire larger sample sizes. However, some of the previous studies did and yet found no connection between immune function and facial attractiveness (Cai et al., 2019; Foo et al., 2017b). Due to conflicting or null results of recent studies investigating links between immune system function and facial attractiveness, Jones et al. (2021) proposed that future research might focus on other qualities to which facial appearance cues, such as an individual's lifestyle health. That is their diet and physical exercise, which can promote more immediate changes in appearance, e.g., through changes in facial colouration (consumption of fruits and vegetables) or weight. As an intersection between focusing on lifestyle health or immune system function, we would suggest investigating whether individuals with varying immune system quality (though we get back to the issue of how to assess immune system quality) display cues to their lifestyle differently. For example, do individuals with a lower immune system quality display more evident signs of an unhealthy lifestyle (e.g., through facial colouration)?

Investigating lifestyle health implies focusing on more immediate changes in appearance. Changes in facial appearance can also be caused by acute illness, which we have likewise explored in this chapter, and one of our studies showed that having an activated immune system is connected to being perceived as less attractive and healthy (Schwambergová et al., under review). It seems that individuals are generally sensitive to cues of illness in others, which is considered adaptive and could be ascribed to the functioning of the behavioural immune system (for review, see Schwambergová et al., 2020). Lastly, in this chapter, we described some of the facial features which play a role in facial attractiveness judgements.

3 FACIAL DOMINANCE

Dominance is a rather complex, multifaceted concept. Dominance in evolutionary sciences is often associated with success in contests or agonistic interactions, which can be achieved by posing harm to rivals or simply intimidating them. The dominant individual can then exclude their rivals from access to mates and resources. Dominant position can often be established based on certain behavioural and morphological traits, e.g., aggressiveness (direct, indirect), larger body size, or other cues to fighting ability (Chen Zeng et al., 2022; Qvarnström & Forsgren, 1998)

Importantly, asserting dominance doesn't always have to be physical in nature. Our understating of dominance should also encompass the non-physical elements, such as "social" skills and tactics utilised to establish a dominant position through coercive authority, intimidation, aggression (verbal, indirect), manipulation and the use of rewards and punishment to influence others (Chen Zeng et al., 2022; Maner & Case, 2016) (which may or may not be physical). This might be important because, as previously shown, women engage less frequently in overt physical encounters than men (Archer, 2004), and resort more often to indirect aggression (presumably to gain a dominant position), such as rival derogation and social exclusion (Campbell, 2015; Vaillancourt, 2013). By fully acknowledging this, we can better account for female-female competition in research.

In this thesis, dominance is often referred to alongside terms such as formidability, masculinity, fighting ability or physical strength. Regarding the connection between dominance, masculinity and physical strength, previous research provides some insights. Perrett et al. (1998) demonstrated that perceived dominance is associated with facial masculinity. Building upon that, Fink et al. (2007) showed a positive association between hand grip strength and both perceived facial masculinity and dominance. Formidability is sometimes referred to as "physical dominance" (Richardson et al., 2021) and is tied to factors such as physical size and strength (Fessler et al., 2012). While studies suggest the interconnectedness of these attributes, we suppose that they are not synonymous. This seems to be the case, for example, when social and physical dominance are distinguished in studies (Quist et al., 2011; Watkins et al., 2010, 2012) and also when facial features connected with the perception of the individual characteristics are investigated as there seems to be some overlap but not absolute (Windhager et al., 2011). However, it is challenging to draw clear lines between individual concepts of dominance, formidability or masculinity. It also seems that there are not enough studies that would focus on one concept in both sexes (e.g., dominance), and if we chose to report studies using only one concept here, it might result in omitting important evidence. We aim to navigate this by stating specific terms used in respective studies when reporting their results. In our own studies, we used the term formidability for judgements of only male faces (as we were interested directly in it) and dominance for judgements of both male and female faces. We believed that formidability, due to its main connection with physical encounters, physical strength and fighting ability, might not be the most appropriate characteristic for assessing female faces. This was also true for masculinity, which might be a problematic concept for participants, especially when rating female faces. We speculate that the term could be either confusing or, on the other hand, too semantically loaded for use with participants in our studies. Dominance, on the other hand, appeared to better encompass both the physical and non-physical aspects (Dong et al., 2023; Watkins et al., 2012).

In the previous chapters, we referred to traits of formidability and dominance, which are considered cues to an individual's success in competition and confrontations (Třebický et al., 2012). Sensitivity to these cues is important for the individual, as it can help them determine whether to engage in direct conflict and/or whether the potential risks (e.g., injury, loss of resources) are worth it. There are numerous proposed dominance and/or formidability cues, but regarding faces, those have been suggested to be likewise morphological (e.g., facial masculinity, facial width-to-height ratio (fWHR), specific development of facial features) and non-morphological (e.g., facial colouration) (Fink et al., 2007; Puts, 2010; Stephen et al., 2012; Třebický et al., 2013).

3.1 Dominance and formidability measures and perception

As there is evidence that certain facial traits are perceived as dominant, formidable or masculine (Keating, 1985; Stephen et al., 2012; Vernon et al., 2014), one of the challenges that researchers face is how to assess an individual's actual formidability, to investigate whether there is a connection between the two. As studying outcomes of real physical conflicts is largely problematic, many of the studies used measures such as body strength (and mostly upper body) and body size for the estimation of an individual's actual dominance and formidability (Fink et al., 2007; Puts, 2010; Sell et al., 2008). It was shown that the hand-grip strength (a measure of upper-body strength) of men positively correlated with ratings of facial masculinity and dominance (Fink et al., 2007). Furthermore, "actual threat potential" (hand-grip strength, height and weight of men) was positively associated with "facial threat potential" (perceived facial dominance, strength and weight judged by women) (Han et al., 2017). By the latter study, we are getting to an important note that measured body size or strength might be connected to actual formidability; however, these two measures are not assessing formidability per se. Instead, these measures correspond more to the "threat potential", which or might not be translated to how the individual would be successful in a physical encounter.

As a result, researchers directed their attention towards acquiring data about mixed martial arts (MMA) contests and their outcomes. Třebický et al. (2013) found that the perceived aggressiveness of MMA fighters in facial photographs was positively associated with fighters' fighting ability (measured by win-loss ratio). Further, participants could correctly identify winners of MMA competitions, and winners were also perceived as more masculine, more aggressive, and stronger than the losers (Little et al., 2015). On the other hand, in the sample of MMA fighters, boxers, and kickboxers, no significant association between perceived facial aggressiveness and fighting success was found (assessed by wins, losses and draws, which is a departure from how the scores and thus the fighting ability was assessed in previous studies) (Richardson et al., 2021) and another study also showed no association between actual and perceived fighting ability (Třebický et al., 2019). Another line of studies investigated perceived facial dominance and its association with performance in dyadic face-to-face competitions (arm wrestling, able pinball soccer, snatching game and verbal fluency game) in the student population and showed positive associations between perceived facial dominance and arm wrestling (mediated by physical strength) (Kordsmeyer et al., 2019).

In women, actual dominance has been mostly assessed by questionnaires (though questionnaires are also used in research on men's dominance (Lefevre et al., 2014; Mileva et al., 2014; Watkins et al., 2010). Women scoring higher on dominance (ostensibly index of social dominance) were perceived as more masculine. Additionally, prototypes of average facial characteristics of women scoring high on dominance questionnaires were perceived as more masculine than those with lower scores (Quist et al., 2011). Not omitting the possible physical components of dominance, Muñoz-Reyes et al. (2012) found that self-perceived fighting ability was positively associated with hand-grip strength in adolescent women, and, in turn, self-perceived fighting ability was positively associated with physical aggressiveness (measured by questionnaire) in middle adolescent women, but the connection disappeared in late adolescence. It has been further shown that facial masculinity (measured by GMM), but not perceived facial aggressiveness and dominance (Gallup et al., 2010), positively correlated with hand-grip strength in women (Van Dongen, 2014).

3.2 Dominant and formidable facial features

Throughout this chapter, we have referred to facial traits that are considered dominant or formidable in faces. One of those can be facial masculinity. As seen above, facial masculinity shows a positive association with perceived dominance both in men (e.g., Albert et al., 2021; DeBruine et al., 2006; Hill et al., 2013; Perrett et al., 1998) and women (Perrett et al., 1998; Quist et al., 2011; Watkins et al., 2012). Some of the studies also distinguish between physical (likely winning in a fistfight) and social dominance (telling other people what to do, respected, influential, and often a leader) when investigating facial masculinity and show interesting variations. Masculinised male and female faces were perceived as physically more dominant and also more socially dominant in the case of male targets, as judged by male raters (Watkins et al., 2010). Conversely, feminised female faces were perceived as more socially dominant than their masculinised versions. This suggests an overlap between the constructs of physical and social dominance in male but not in female targets (Watkins et al., 2010). In another study, which also distinguished between social and physical dominance, masculinised versions of female faces were perceived as more physically dominant than the feminised versions (Watkins et al., 2012). Interestingly, feminised versions were perceived as more socially dominant, replicating the results of (Watkins et al., 2010), this time with female raters (Watkins et al., 2012). However, study methodologies may play an important role. A recent study (Dong et al., 2023) showed that facial masculinity was strongly connected with perceived dominance when computer-manipulated stimuli were used (their feminised or masculinised versions) in a forced-choice test – and this methodology was also used in some of the studies presented above (DeBruine et al., 2006; Perrett et al., 1998; Watkins et al., 2010, 2012). The study also showed that when using design with sequential presentation and rating of unmanipulated (natural) stimuli, masculinity wasn't often significantly associated with perceived dominance, and if it was, the effect sizes were substantially smaller in comparison to forced-choice test with manipulated stimuli (Dong et al., 2023).

Another morphological trait of interest is the facial width-to-height ratio (fWHR), which is measured by dividing facial width by facial height. fWHR is proposed to be associated with testosterone levels during puberty, i.e., individuals with higher testosterone levels are expected to have higher fWHR (Lefevre et al., 2013; Verdonck et al., 1999) and higher testosterone levels were linked to aggressive behaviour (Archer, 2006). Higher fWHR was found to be connected with self-reported dominance and physical, verbal aggression and anger in both men and women (Lefevre et al., 2014; but see Lefevre & Lewis, 2014). Further, fWHR was positively associated with other-perceived aggression in both sexes, though the association was stronger for men (Lefevre & Lewis, 2014). It was also associated with other-perceived dominance, but only in men (Mileva et al., 2014) and predicted fighting success (winners in MMA fights) in men (Caton et al., 2022; Třebický et al., 2015; Zilioli et al., 2014).

Morphological studies also highlighted specific facial features and their appearance related to facial dominance, formidability, and masculinity. In men, features connected with perceived dominance were a prominent square jaw, thick eyebrows, small eyes, and thin lips (Keating, 1985). More recent studies using GMM methods showed that features connected with perceived facial dominance and masculinity largely overlap, and those are smaller eyes, shorter nose, and wider and more prominent lower jaw (Windhager et al., 2011), and in another study, as masculine were also perceived shape features such as wide nose, thin lips, and wider inter-orbital distance (Mitteroecker et al., 2015). Perceived aggressiveness has been shown to be connected with facial features such as a broader chin, larger nose, deep-set eyes and prominent eyebrows (Třebický et al., 2013). Moreover, authors described men's facial features linked with higher hand-grip strength, and those were larger eyes that were further apart, higher and thinner eyebrows, narrow mouth and less prominent chin in relation to the rest of the lower face (Windhager et al., 2011). Features connected with actual fighting success were a narrower chin, bigger nose, and mouth (Třebický et al., 2013).

In women, Keating (1985) showed that the combination of a prominent square jaw, thick eyebrows, smaller eyes, and thinner lips was positively associated with dominance ratings by others. However, more studies investigating specific facial features connected with women’s dominance (either other- or self-perceived), and especially those using GMM methods, are, to my best knowledge, missing. To obtain more evidence, we can turn to those studies that showed that female facial masculinity increases perceived dominance (Quist et al., 2011; Watkins et al., 2010, 2012) and features corresponding to facial masculinity are described as, e.g., having a more prominent nose and supraorbital arcs, wider jaw, and smaller eyes (Fink et al., 2005; Gangestad & Thornhill, 2003).

In this section, we first briefly discussed individual concepts such as dominance, formidability, masculinity, fighting ability, and their possible connection. We showed that studies indicate some links between them, and some even use them almost synonymously, especially when focusing on male targets. To not omit important evidence if we chose to focus only on one concept, we decided to report the results of those studies incorporating relevant concepts here and state the exact terms the studies used. We also proposed the concept of dominance as being better suitable when focusing on intrasexual competition in both male and female targets, while formidability is probably more sensible for use mostly in male targets. We have seen that in men, there is increasing evidence that facial characteristics cue actual fighting ability and performance in physical competitions (e.g., Kordsmeyer et al., 2019; Little et al., 2015; Třebický et al., 2015). In women, the connection between facial characteristics and actual dominance is largely missing. We have discussed the most often researched specific facial morphological traits (masculinity, fWHR) and described facial features and their appearance, which relate to perceived or actual formidability and dominance, but mostly in men (Windhager et al., 2011). Moreover, it seems that facial features and their appearance related to the perception of male dominance, masculinity, aggression, or formidability partially overlap in some instances yet differ in others. For instance, thin lips were connected with perceived dominance and masculinity but not with perceived aggressiveness or actual fighting success. This further implies that the individual concepts are related but not synonymous. The evidence for women is likewise largely missing, though it seems that perceived dominance in women is often linked to masculine facial features (e.g., wider jaw, smaller eyes) (Quist et al., 2011).

4 EYE-TRACKING

There is substantial evidence that both men and women attribute attractiveness and dominance or formidability to faces, and numerous studies identify specific morphological facial features that play a role in the judgements (Little, 2014; Třebický et al., 2013; Windhager et al., 2011). However, little is known about whether individuals pay direct visual attention to those features and, if so, which features are the most visually attended ones. Moreover, one can ask whether specific judgement would affect which facial features would be directly visually explored and whether the rater's sex plays a role. Further, a gap exists in understanding how the level of dominance or formidability affects visual attention towards faces and facial features and whether this effect would be likewise sex-specific.

Although methodologies based on explicit stimuli ratings used by most studies in this field of research yield invaluable data, they remain susceptible to biases stemming from participants' self-reported information, which may be influenced by their individual beliefs and social desirability tendencies (Holtgraves, 2004). One of the methods that is arguably not as commonly used in this area of research but enables direct investigation of autonomous visual attention and directions of eye gaze while avoiding self-reporting is eye-tracking.

Eye-tracking allows insight into autonomous visual attention processes. It can identify the direction and length of eye gaze, i.e., where exactly the participants are looking at the visual stimuli, which is usually connected to the definition of areas of interest (AOI). Various metrics related to visual attention can be collected, such as fixations (fixation occurs when the gaze rests on a particular location, incl. AOI) or saccades (quick eye movements from one visual target to another). Here, we will more closely focus on number of fixations, mean fixation duration, i.e., how long the fixation is on average, dwell time in AOI, which sums the time spent looking at AOI, or visit duration in AOI, which is the time between the first fixation in AOI and the next one outside. Visit duration also includes saccadic duration between the fixations inside the AOI. If one uses the sum of all visit durations to estimate (overall) visit duration in the AOI, then it is a rather similar metric to dwell time; the difference between them is that the first one includes the saccadic duration while the latter doesn't. It seems that which metrics are employed in individual studies largely depends on the study design, researcher choice, as well as on the eye-tracking system which is used. The above-discussed metrics are arguably the most commonly used in this area of research, as they are well-suitable for detecting visual attention to a specific target (Skaramagkas et al., 2023) and were also used in our studies. There are, of course, numerous other metrics eye-tracking enables collecting, e.g., saccades, blinks, pupil size variation and more (Duchowski, 2017).

The metrics described above refer to sometimes different, though often overlapping, aspects of cognitive processes behind visual attention. The higher number of fixations is thought to be connected with the importance of the stimuli or their areas (Jacob & Karn, 2003). However, it can also indicate the informativeness of the visual stimuli and their liking (Goller et al., 2019) or can also correspond to the cognitive load they impose (Duchowski, 2017; Skaramagkas et al., 2023). Fixation duration is generally considered to be positively associated with task difficulty (Galley et al., 2015). Dwell time and visit duration have been, similarly to the number of fixations, connected with importance, informativeness or liking of the stimulus, and task difficulty (Duchowski, 2017; Jacob & Karn, 2003; Shimojo et al., 2003).

Several aspects of the eye-tracking methodology should raise our attention. One of them is that defining AOI is based on the judgement and scientific questions of the researcher, and studies tend to differ in their number, placement, shapes and sizes, even when they are inspecting similar topics. This can then significantly affect the results and comparability of the studies (Hessels et al., 2016). Moreover, studies sometimes use and interpret individual eye-tracking metrics interchangeably, even though they can correspond to different cognitive processes (Skaramagkas et al., 2023). However, sometimes it is difficult to differentiate between the cognitive processes based on the eye-tracking metrics, as for many of the metrics, they overlap (Skaramagkas et al., 2023). Consequently, it can be challenging to distinguish whether heightened fixation on some area corresponds, e.g., to its informativeness, importance or liking. However, this would also highly depend on the research questions and design. For some research, this will not be a substantive issue, i.e., in research focusing on solving intelligence tests, the prolonged looks will more likely be caused by the task difficulty than by its liking.

4.1 Eye-tracking and judgements of facial attractiveness and dominance

Many studies employing eye-tracking in face research have primarily focused on recognition and memory (Althoff & Cohen, 1999; Barton et al., 2006; Millen et al., 2017; Millen & Hancock, 2019) or emotions (Asthana & Manual, 2001; Hall et al., 2010). Others employed a free-viewing paradigm, which is when participants are instructed to freely look at certain stimuli without any other specific task (Hickman et al., 2010; Semmelmann & Weigelt, 2018). When participants are looking at faces during the free-viewing task, the eyes, nose, and mouth usually attract visual attention the most (Hickman et al., 2010; Król & Król, 2019;

Semmelmann & Weigelt, 2018). Fewer eye-tracking studies focused on visual attention to faces across different contexts, let alone compared the visual attention to faces during the judgements of facial attractiveness and dominance. Focusing on these contexts would be highly valuable for research on intersexual and intrasexual selection, which are of interest to evolutionary sciences.

A study examining visual attention to female faces during attractiveness judgements found that men and women looked the longest at the nose and then at the eyes and lips (Zhang et al., 2017). In another study that assessed visual attention to male and female faces during judgements of attractiveness and age, the task didn't influence visual attention, with the eyes and nose receiving the most fixations (Kwart et al., 2012). Similarly, a study investigating differences in visual attention to faces during judgments of dominance and trustworthiness likewise showed that the visual attention towards individual AOIs didn't differ between tasks and that the eyes, nose and mouth received the most visual attention (Hermens et al., 2018). The studies showed similar results regardless of whether only AOIs specified were the eyes, nose and mouth (Zhang et al., 2017) or when including more areas (e.g., chin, forehead) (Hermens et al., 2018; Kwart et al., 2012).

As there is not enough evidence regarding visual attention to faces in judgements relevant to intersexual and intrasexual selection, in our study, we explored the visual attention (number of fixations, fixation duration, visit duration) to the faces of potential partners (opposite-sex assessment of attractiveness) and rivals (same-sex assessment of dominance). We defined a broader array of AOIs based on insights from prior studies (e.g., using GMM), which suggested relevant facial features for respective judgements (see Chapters 2, 3 and 8).

It seems that for women, partner choice may be more important, as potential partners' faces receive more visual attention than potential rivals. However, women, compared to men, looked longer on all faces regardless of context, which might indicate that women were generally more engaged in the tasks, or both tasks might be important for them. Besides the already proposed importance of partner choice for women, the fact that women looked more than men at potential rivals might indicate that it could be a more challenging task for them, as they might not be used to judging other women's dominance. It is possible they may have been trying to estimate dominance through other characteristics (which resulted in longer looks), such as attractiveness, as women were proposed to compete in terms of attractiveness (Fisher, 2004;

Puts, 2010). Another possible explanation might be that they have been directly comparing themselves to the stimuli, which might have prolonged the looks as well.

Same as in previous studies, the most looked at facial features across the contexts (potential partners and rival rating) and sexes were the eyes, nose and mouth. Further, studies highlighting jaw and cheekbones as important in judgements of male attractiveness (Cunningham et al., 1990; Windhager et al., 2011) might be somewhat supported by our study, as women looked longer at the chin and left cheek when assessing the attractiveness of potential partners. Being attentive to these features could also be crucial because of their possible connection to male formidability and its link to both beneficial and unfavourable characteristics in a partner (Qvarnström & Forsgren, 1998; Třebický et al., 2012). Moreover, in men, the chin of potential rivals received more visual attention than that of potential partners, corroborating the feature's importance in male intrasexual competition (Windhager et al., 2011). Some support for the importance of nose and mouth ratings of female attractiveness by men was found, which is in line with previous studies (Abend et al., 2015; Pflüger et al., 2012). For more detailed results and discussion, see Chapter 8.

Another question to consider is whether the differences in the level of the target's facial attractiveness or formidability and dominance might also play a role in visual attention to faces and their features. Studies demonstrated that more attractive faces generally attract more visual attention (Kwart et al., 2012; Maner et al., 2003), and the preference for and heightened visual attention to attractive traits seems to appear early in ontogeny (Griffey & Little, 2014). Furthermore, the positive association between the target's facial attractiveness and the rater's higher visual attention in adults tends to be generally stronger for the preferred sex (Leder et al., 2010; Mitrovic et al., 2018; Valuch et al., 2015). Notably, higher facial attractiveness captures visual attention across various attractiveness levels, not just when a very attractive face is displayed or when the faces displayed side by side substantially differ in attractiveness (Leder et al., 2016). However, for men looking at other men with different levels of facial attractiveness, the heightened attention towards attractive faces wasn't as pronounced (Leder et al., 2016; Mitrovic et al., 2018) or wasn't found at all (Mitrovic et al., 2016).

Whether there is a variation in which facial features attract visual attention in relation to the level of attractiveness was inspected in a study by Kwart et al. (2012). They found that the nose and mouth of attractive faces were fixated more often than unattractive faces. While they say that the differences in the number of fixations were quite small, they interpret it so that while eyes are predominantly fixated across judgements and faces with different characteristics, nose and mouth might be particularly important for attractiveness judgements. Consequently, while we have some evidence about how different levels of facial attractiveness influence visual attention towards faces, this is not the case for formidability.

In our study, we assessed the visual attention of men and women, using a number of fixations and dwell time, to male faces and their features when judging attractiveness and formidability. Moreover, we investigated the effect of the target's level of attractiveness and formidability (low, medium, high) on the rater's visual attention (Chapter 9). Raters, irrespective of sex, looked longer towards faces with a medium level of formidability than a high level. This might be due to the ambiguity of the faces and the uncertainty of how to rate them (Martín-Loeches et al., 2014). We found that men looked longer on the male faces than women during both attractiveness and formidability assessments. This could suggest task difficulty for men, possibly arising from direct self-comparison with the stimuli. In our previous study (Pátková et al., in revision), we observed the opposite - women gave more visual attention to faces. The study designs slightly differed, for e.g., one study used only male faces as stimuli, and the other used both male and female faces, which makes the direct comparison in this case challenging. What can be concluded is that it doesn't seem to be the case that one sex would generally be more visually attentive to whole faces in intersexual and intrasexual selection, but the context, the target's sex or their interaction play a role.

We haven't demonstrated that individuals would look more towards faces with a higher level of attractiveness. For men, this might be partly explained by direct comparison between themselves and the stimuli, therefore needing similar time to extract the relevant information, no matter the target's attractiveness (Mitrovic et al., 2016). Why we haven't found any effect even for female raters (though this might be an alternative explanation also for male raters) might be due to different stimuli presentation to other studies, i.e., in our case, sequential where raters saw one face at a time, but some of the previous studies presented two faces side by side (e.g., Leder et al., 2016; Mitrovic et al., 2016). The variability in the target's attractiveness/formidability might also influence the detectable visual attention differences; for more on this, see Chapter 9.

The eyes, nose and mouth received by far the most visual attention during judgements of male attractiveness and formidability (Pátková et al., submitted). As in our previous study (Pátková et al., in revision), we observed variation in visual attention towards the chin. In that study, men directed their visual attention more towards the chin of potential rivals (male faces) than partners (female faces). In this study (Pátková et al., submitted), men visually attended chin on male faces more than women. It might suggest that the chin is generally important for male-male attractiveness or formidability judgements, though we remain cautious in our interpretations as the sexes of the stimuli differed between the studies. Given that men, compared to women, directed more visual attention towards cheeks and forehead during formidability judgements, it might suggest their importance in intrasexual selection. The cheeks AOI also covered part of the jaw, which has been associated with facial formidability judgements (Keating, 1985; Windhager et al., 2011). Further, the forehead may offer cues about an individual's strength (Windhager et al., 2011), and a smaller forehead is connected to facial masculinity (Komori et al., 2009), though this area is not commonly highlighted as important for formidability judgements in other studies.

We suggest that if features such as cheeks and chin played a significant role in intersexual and intrasexual selection, they would receive more visual attention than they did (Pátková et al., submitted, in revision). We speculate that they might be directly visually attended only when an individual is still unsure about the judgement, after inspecting the most important areas – eyes, nose and mouth, or when the feature specifically attracts attention, arguably because of unexpected shape. For more detailed results and discussion, see Chapter 9.

Another important point is that although specific features may not have been the direct focus of visual attention, it doesn't mean participants didn't perceive them at all. The central vision area probably covered multiple features (AOIs) simultaneously (see Pátková et al., submitted). Consequently, participants might have processed features which weren't directly fixated, and eye-tracking may not fully capture this. That attractive faces can be detected well in parafoveal (area with lesser visual acuity) and even in peripheral vision was previously shown (Guo et al., 2011).

In this chapter, we discussed the direct visual attention towards faces and their features in general and also under specific contexts or judgements. Numerous morphological studies identified various facial features as important to judgements of facial attractiveness, dominance or formidability (Cunningham, 1986; Little et al., 2011; Pflüger et al., 2012; Windhager et al., 2011). Moreover, a limited number of studies assessed changes in visual attention to faces under different contexts (but see Hermens et al., 2018; Kwart et al., 2012). In our eye-tracking studies, we investigated visual attention patterns during mate choice and competition contexts

(Chapters 8 and 9). Consistent with prior eye-tracking research (Hermens et al., 2018; Kwart et al., 2012), the eyes, nose and mouth consistently emerged as the most visually attended facial areas across sexes and contexts, though some variation was found (Pátková et al., in revision, submitted). Further, we demonstrated variation in visual attention towards some features suggested by morphological studies as important in respective judgements, such as cheek and chin. However, the visual attention towards these areas was rather small (Pátková et al., in revision, submitted), and even though we interpreted it, we remain cautious about how much practical importance they carry. Consequently, regardless of the assessed characteristics, it appears that evaluations primarily rely on the position, shape and development of eyes, nose, and mouth, and we argue these features are central for acquiring information about others. Further, while some studies demonstrated that more attractive faces capture increased visual attention (Leder et al., 2016; Mitrovic et al., 2018), we did not observe this relationship. This could be attributed to various factors, such as the different design of our study, i.e., sequential presentation of stimuli or limited variability in the stimuli (Pátková et al., submitted). The visual attention towards formidable faces in relation to their formidability level hasn't been previously investigated, and we showed that faces with a medium level of formidability than those with a high level receive more visual attention (Pátková et al., submitted). To sum up, in the two of our eye-tracking studies, we have inspected visual attention towards faces and their features and shed more light on visual attention across different judgements related to intersexual and intersexual selection.

5 THE RELATIONSHIP BETWEEN INDIVIDUAL MODALITIES

Throughout this thesis, we focused on visual modality, which is arguably the most studied modality in the context of intersexual and intrasexual selection, as humans generally rely heavily on their sense of vision. However, people assess others not only via visual perceptual cues and signals but employ multiple senses (Candolin, 2003) and are capable of judging attractiveness and/or masculinity, dominance or formidability also from human body odour (Fialová et al., 2020; Havlicek et al., 2008; Rikowski & Grammer, 1999) or voices (Borkowska & Pawlowski, 2011; Schwambergová et al., under review; Valentova et al., 2017). A certain level of error can accompany judgements relying on a single sensory modality. As a result, using multiple sensory modalities could lead to a more reliable assessment. There are two main hypotheses which try to explain how multiple modalities are used in individual judgements. The "backup" signals hypothesis proposes that individual cues provide overlapping and redundant information because they reflect the same quality. Using backup cues should aid in reaching higher accuracy in assessing mate quality while also making it harder for the potential mates to "cheat" about their quality (Candolin, 2003; Grammer et al., 2002). The "multiple messages" hypothesis says that different cues provide information about different individual's qualities and that the cues are therefore non-redundant (Moller & Pomiankowski, 1993). Focusing on various cues together can then provide information about the general quality of the individual, or assessed individually, they can provide information about its specific aspects (Candolin, 2003). This would also allow individuals to be selectively attentive towards cues and aspects of individual mate quality, perhaps in relation to their own condition or genetic information (Wedekind, 1997). Consequently, if the assessments of certain traits via different modalities are strongly positively associated, it should suggest support for the backup signals hypothesis, while if no or only a weak association between them is present, this lays support towards the multiple messages hypothesis (Candolin, 2003). One of the issues lies in the lack of clarity regarding the strength of the association between modalities necessary to substantiate either hypothesis, i.e., which association should be considered weak or strong in this context.

In two of our studies, we found support for the "multiple messages" hypothesis. We showed that the faces of individuals with an activated immune system were rated as less attractive and healthy, but the body odour of these individuals was rated as more attractive. Furthermore, no changes in perceived vocal attractiveness were detected (see Chapter 7). As under the "back up" signal hypothesis, we would expect the shifts in attractiveness judged via different modalities to follow the same direction, it seems that in our case, they conveyed information about different aspects of individuals' condition (or quality). Shortly after immune system activation, there is a documented negative change in body odour (Olsson et al., 2014). However, currently, we have no data on how long this effect persists; a repeated body odour collection in intervals would be able to answer this question. In our study, the stimuli were collected two weeks after vaccination, when we expected the highest increase in antibodies. However, we speculate that within this time frame, the negative effect of immune system activation might pass, allowing body odour to return to its baseline quality. This return to baseline may enhance the positive shift in body odour attractiveness at a certain point, which might then cue the quality of an individual capable of effectively dealing with illness. On the other hand, the negative effect of vaccination on facial attractiveness might be perhaps due to the lingering effect of the immune system activation, still being perceivable through visual cues. The immune system activation is energetically demanding, possibly manifesting for a longer period as a tired appearance, which is closely associated with looking ill (Axelsson et al., 2018). Moreover, it might be beneficial if individuals on the brink of recovery would be perceivable at least by some modality (e.g., rather to avoid already non-contagious individuals than to risk becoming infected).

In the second study, we performed a systematic review and meta-analysis of both published and unpublished studies to inspect the concordance in judgements of body odour and facial attractiveness and body odour and vocal attractiveness. For the relationships between body odour and facial attractiveness and body odour and vocal attractiveness, we found weak positive associations. Due to the weakness of the associations, the results would rather support the “multiple messages” hypothesis, that is, body odour, facial appearance, and voices provide non-redundant information about an individual’s mating quality (Třebický et al., 2023). These results align with other animal studies (Candolin, 2003; Kraak et al., 1999), though they are in contrast with some previous studies that observed a stronger correlation between facial and body odour attractiveness (Mahmut & Stevenson, 2019; Rikowski & Grammer, 1999; Thornhill et al., 2003). Further, our results might also be somewhat surprising in the context of other studies which showed that facial attractiveness is influenced by specific traits and features (Chapter 2), which should cue the individual’s quality, e.g., health and developmental stability (see Chapters 2, 6 and 7). Similarly, body dour has been suggested to convey information about an individual’s quality (among others, likewise health and developmental stability) (Havlíček et al., 2017). This would suggest at least moderate associations between the two modalities, but we found only weak ones. Nevertheless, a lingering question remains whether individual modalities indeed cue an individual's qualities (and which ones), as more recent research often lacks evidence for such links (Jones et al., 2021; Stephen & Luoto, 2023) and Chapter 6.

The relationship between facial and vocal attractiveness is yet to be thoroughly investigated, and studies showed mixed results in support of either of the hypotheses (Smith et al., 2016; Valentova et al., 2017), while others suggest that the effects might be sex-specific (Valentova et al., 2017; Williams & Lee Apicella, 2023). The latter study also found that in men, the judgements of attractiveness across modalities weren't correlated (therefore supporting the "multiple messages" hypothesis). In women, they found weak correlations between modalities (and interpreted it in favour of the "back up signals" hypothesis) (Williams & Lee Apicella, 2023). This might point back to the issue that it is not clear how strong the association between modalities should be to corroborate one hypothesis or the other. For more on this, see Chapter 10.

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PART II.

Chapter 6

ATTRACTIVE AND HEALTHY-LOOKING MALE FACES DO NOT SHOW HIGHER IMMUNOREACTIVITY

scientific reports

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Attractive and healthy-looking male faces do not show higher immunoreactivity

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Previous research has indicated that facial attractiveness may provide cues to the functioning of the immune system. Mating with individuals who have a more effective immune system could lead to a higher reproductive success. Our main aim was to test a possible association between immunoreactivity (stimulated by vaccination) and perceived facial attractiveness and healthiness. We experimentally activated the immune system of healthy men using vaccination against hepatitis A/B and meningococcus and measured levels of specific antibodies (markers of immune system reactivity) before and 30 days after the vaccination. Further, 1 day before the vaccination, we collected their facial photographs that were judged by females for attractiveness, healthiness, and facial skin patches for healthiness. In view of its proposed connection with the functioning of the immune system, we also measured skin colouration (both from the facial photographs and in vivo using a spectrophotometer) and we assessed its role in attractiveness and healthiness judgements. Moreover, we measured the levels of steroid hormones (testosterone and cortisol) and the percentage of adipose tissue, because both are known to have immunomodulatory properties and are related to perceived facial attractiveness and healthiness. We found no significant associations between antibody levels induced by vaccination and perceived facial attractiveness, facial healthiness, or skin healthiness. We also found no significant connections between steroid hormone levels, the amount of adipose tissue, rated characteristics, and antibody levels, except for a small negative effect of cortisol levels on perceived facial healthiness. Higher forehead redness was perceived as less attractive and less healthy and higher cheek patch redness was perceived as less healthy, but no significant association was found between antibody levels and facial colouration. Overall, our results suggest that perceived facial attractiveness, healthiness, and skin patch healthiness provide limited cues to immunoreactivity, and perceived characteristics seem to be related only to cortisol levels and facial colouration.

Mate preferences are often based on physical appearance, whereby facial attractiveness seems to play an especially significant role[1]. It is often claimed that facial attractiveness provides cues to various aspects of individuals' quality, such as immunocompetence[1–3]. Selection of partners with a more effective immune system is expected to lead to a higher reproductive success by passing increased pathogen resistance onto the offspring (indirect benefits). Moreover, healthier individuals can provide better parental care and are less likely to transmit any infections to their partners (direct benefits)[1,4].

Previous research into the putative relationship between facial attractiveness and individual's quality that was conducted using self-reported past and current health and attractiveness ratings of facial photographs delivered mixed results[5,6]. Several recent studies employed direct immunity function measures, such as inflammation markers or levels of cytokines or antibodies. In a sample of South African men, Phalane et al.[7] tested the relationship between facial attractiveness ratings and responsiveness of the immune system upon activation by an injection of bacterial lipopolysaccharide (LPS). Immune system response was assessed by levels of C-reactive protein, which is an inflammation marker, and by the levels of cytokines, which are peptides that stimulate the immune response. This study found a positive correlation between facial attractiveness ratings and the levels of cytokines, specifically interleukins (IL)-2, 4, 6, 8, and 10, granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon γ (IFN-γ), and tumour necrosis factor α (TFN-α)[7]. Other studies employed vaccinations to elicit and measure immune system reactivity. A stronger response to the vaccine (assessed via higher antibody levels) indicates a

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better protection against infection[8]. Overall, though, the results of these studies are inconclusive. Faces of men with higher levels of hepatitis B antibodies were rated as more attractive[9] but this did not hold of women[10]. In contrast, other study reported a negative, though nonsignificant, association between facial attractiveness and immune system reactivity in men[11].

It has been suggested that facial skin colouration plays an important role in perceived facial attractiveness and health[12–15]. Studies tend to focus on facial skin colouration in the CIE L*a*b* colour space[16,17]. Higher skin redness (a*) is linked to increased skin blood perfusion and oxygenation[16], which are in turn positively associated with physical fitness[18,19] as well as good cardiovascular[20] and pulmonary health[16,18]. Skin yellowness (b*) is influenced by carotenoids, which are pigments acquired from food, mainly fruits and vegetables. Owing to their antioxidant properties[21], carotenoids can contribute to disease resistance as they can destroy free radicals and reduce oxidative stress, both of which are harmful to the immune system[22,23]. It has been shown that facial skin with higher redness and yellowness is perceived as more attractive and healthier[17,24]. Moreover, Phalane et al.[7] reported an association between skin yellowness and a marginally higher immune system response (higher levels of inspected cytokines) after LPS stimulation. On the other hand, Foo et al.[25] found that higher skin yellowness is positively associated only with perceived health (and only in men) and not with direct immune function measures[25]. Skin lightness (L*) is determined by the distribution pattern of melanosomes in keratinocytes and the amount of melanin it contains[26]. Higher melanin levels (resulting in a darker skin hue) can provide a better protection against sunlight[27] but can also contribute to vitamin D deficiency[28]. It has been found that melatonin can have an effect on the synthesis of melanin[29], which is in turn believed to affect the periodicity of immune response as well as cytokine production[30,31]. In women, lighter skin is associated with higher perceived attractiveness and youth[32–34] (but see Fiala et al.[35]), because with increasing age skin tends to become darker[36]. In men, some research shows that darker complexion may be preferred[37].

The association between functioning of the immune system and perceived facial attractiveness might be also modulated by testosterone and cortisol. It has been suggested that testosterone has an immunosuppressive effect[38–40] but evidence to that effect is rather mixed[41]. It has thus been proposed that glucocorticoids, such as cortisol, mediate the association between testosterone and the immune system functioning[42,43]. Although a short-term elevation of cortisol levels can boost an acute immune system response, prolonged exposure may weaken the response, thereby increasing susceptibility to diseases[44]. Some support for the mediating effect of cortisol comes from Rantala et al.[9] who found that immunoreactivity was stronger in men with higher testosterone and simultaneously lower cortisol levels, while immunoreactivity was also positively linked to facial attractiveness. Similarly, women with lower cortisol levels were perceived as more attractive[10,45] (for null results see Han et al.[46]).

Another key factor affecting both attractiveness and immunity is adiposity. Obesity contributes to an altered immune function and reduced immunocompetence because it is associated with changes in leucocyte counts, reduced antibody production, impaired wound healing, a higher risk of infections, and even a higher mortality rate[47–50]. In perception studies, body fat levels affect attractiveness ratings, whereby both overweight and excessively thin individuals are perceived as less attractive[5,10,51]. Moreover, portrait photographs of individuals with elevated levels of leptin—a hormone produced by the adipose tissue that has a negative effect on health—were also perceived as less attractive[52].

Overall, evidence pertaining to links between the quality of the immune system and facial attractiveness is ambiguous. Many previous studies investigated only a limited number of relationships between variables and relied on indirect measures of immune system functioning. In Study 1, we therefore focused on the relationship between immune system reactivity and perceived facial attractiveness. To measure the reactivity of the immune system, we experimentally activated the immune system by vaccination against both viral (hepatitis A, B) and bacterial (meningococcus) infections, because the two in conjunction should stimulate a wider range of components of the immune system than either would. We used differences in antibody levels before and after vaccination as a proxy for reactivity of the immune system. In Study 2, we investigated associations between immune system reactivity and perceived skin patch healthiness to examine human ability to judge characteristics from limited amount of information. Finally, in Study 3 we focused on the relationship between immune system reactivity and perceived healthiness of the face. Moreover, we measured testosterone and cortisol levels and recorded body composition, because all these factors have immunomodulatory properties and are linked to both perceived facial attractiveness and healthiness. We also measured facial skin colouration (both from the facial photographs and in vivo using a spectrophotometer) to assess its role in attractiveness and healthiness judgements and its connection to the immunoreactivity.

Materials and methods

Data used for this study are part of a larger project which investigates possible associations between reactivity of the immune system and attractiveness of human body odour[53], face, and voice as perceived by opposite-sex individuals. The present article focuses on associations between immune system reactivity and perceived facial attractiveness, healthiness, and skin healthiness. All procedures were conducted in accordance with the Helsinki Declaration and the study was approved by the Institutional Review Board of Charles University (approval no. 20/2016). Due to the nature of this study, we have collaborated with medical personnel. The study was preregistered prior to data analyses (https://osf.io/69zgc). Before entering the study, all participants were informed about its goals and expressed their consent with participation by signing an informed consent form.

Targets. We have collected data from 21 men (mean age = 26.2 ys, SD = 4.62, age range = 20–35 ys). Requirements for participating in the study were the following: age 18–40 years, good general health, no current use of any medication, non-smokers, and not being vaccinated against hepatitis A, B, or the meningococcus in the past 10 years (e.g., Shepard et al.[54]).

Participants were recruited via social media advertisements (Facebook) and leaflets at university halls of the Faculty of Science, Faculty of Humanities, and Faculty of Physical Education and Sports (all of the Charles University, Prague, Czechia). Participants were vaccinated free of charge and received a reimbursement of 400 CZK (approx. €15) for participation in the whole project as a compensation for their time and potential inconvenience. Targets were the same for all studies described in the present article (Studies 1–3).

Procedure. One day before vaccination, we acquired standardised portrait photographs of the participants. On the day of the vaccination, each participant completed a questionnaire on their medical history and their general health status was examined by a physician to ensure they were eligible for application of the vaccines and not suffering from any current illness or infection. This was followed by the first blood collection (5 ml of venous blood) to assess the basal levels of antibodies (specific immunoglobulins G—IgG and immunoglobulins M—IgM) and C-reactive protein (CRP), which is a marker of inflammation. In none of the participants did the pre-vaccination CRP levels exceed 5.5 mg/l; values below this threshold are considered clinically normal[55], that is, such values do not indicate currently ongoing infection. After the blood collection, the vaccines against hepatitis A/B (Twinrix) and meningococcus (Menveo) were administered. We selected vaccines against both viral and bacterial infections to stimulate different components of the immune system (nonspecific, specific, cellular, and humoral). The second blood collection and second photograph acquisition took place 14 days after vaccination, at a time point when one should expect the highest antibody response[56]. For the current investigation, only photographs taken before the vaccination were used. The last blood collection took place 30 days after vaccination, at a point when a second dose of vaccine against hepatitis (Twinrix) is recommended[57]. Vaccination and first blood collection were performed by a physician, while the remaining two blood samples were collected by phlebotomists at the Prevedig laboratory (https://www.prevedig.cz/) where all samples were subsequently analysed. The procedure and time of blood collections were standardised across participants. To avoid diurnal fluctuations[58] sampling was conducted at 7–8 a.m. We measured and recorded body composition of the participants. Participants also completed questionnaires about their health status during the study and about possible factors that may have influenced their skin colour (e.g., traveling abroad to a sunny destination, use of tanning beds, self-tanning creams, or the consumptions of vegetables and fruits with high levels of carotenoids)[59,60]. This procedure took place on Q4 2017 to minimise possible effects of a suntan.

Vaccine characteristics. To induce an immune system response, we used the Twinrix Adult vaccine against hepatitis A/B and a Menveo vaccine against meningococcus (which prevents meningococcal diseases caused by *Neisseria meningitis* serogroups A, C, Y, and W-135). They can be administered together and are widely used in the Czech Republic. Both were applied intramuscularly, each in one arm.

Laboratory assays. All laboratory analyses worked with the serum or plasma and were performed in a certified Prevedig laboratory. Total level of antibodies against hepatitis A (Anti-HAV) were measured by the Diasorin® Liaison—chemiluminescence immunoassay (CLIA), where a fully automated immunological analyser performs the full processing of samples. We used the corresponding Human S100 CLIA kits. This analysis is based on a radioimmunoassay, where the antigen and paramagnetic microparticle solid phase binds with fluorescent-labelled antibodies and after oxidation–reduction reaction, excessive energy is released in the form of photons[61]. The final photometric measurement and evaluation were done by the analyser.

Antibodies against hepatitis B (Anti-Hbs) were measured based on the same principle as Anti-HAV. It turned out, however, that large percentage of targets either had high levels of Anti-Hbs at the baseline (N = 7) or did not respond to vaccination (N = 5). For this reason, the Anti-Hbs were excluded from further analyses.

Antibodies against the meningococcus (Anti-Mnk) were measured by the fully automated Diasorin® ETI-Max 3000—enzyme immunoassay (ELISA), one of the basic methods of determination of serum antibodies. The method is based on a reaction between an antigen on a special board and antibodies in the patient's serum. Then secondary antibodies are added, which are specially labelled and bind to the primary antibodies with the antigen. A chromogenic substrate, which is added last, causes a colour response that is measured by spectrophotometer[62]. Sufficient response is at least 1:4 titres (the dilution of the serum where antibodies still react with the antigens) and ideally even higher[63]. As above, the final photometric measurement and evaluation were conducted by the analyser.

Total testosterone levels were measured by chemiluminescence (CLIA) in a fully automatised analyser Beckman Coulter DxI 800 Immunoassay System. The CLIA principle is described above. In this case, the energy is released by a reaction between testosterone, polyclonal anti-testosterone antibodies, and a tracer[64]. The final photometric measurement and evaluation were done by the automatised analyser.

Cortisol levels were measured by an electrochemiluminescence immunoassay method in a fully automatised analyser Beckman Coulter DxI 800 Immunoassay System. First, one incubates a sample in which specific anti-cortisol antibodies labelled with ruthenium chelate bind to cortisol. This complex is captured on the surface of an electrode where the electric charge causes a chemiluminescent emission of photons. The emitted light is measured by a spectrophotometer, but the measurement and evaluation are likewise done by the analyser.

The acquisition of photographs. Acquisition of photographs took place at the Human Ethology perception lab in a purpose-built photographic booth in order to prevent potential changes in ambient illumination and colour reflections[65].

Portrait photographs were taken with a 24-megapixel full-frame (35.9 × 24 mm CMOS sensor, a 35 mm film equivalent) digital SLR camera Nikon D610 equipped with a 85 mm fixed focal length lens[66] (Nikon AF-S NIKKOR 85 mm f/1.8G) into 14-bit uncompressed raw files (.NEF) and Adobe RGB colour space. The camera was mounted in a portrait orientation directly on a light stand that also carried a strobe light. A single 400Ws studio strobe (Menik MD-400Ws) was used and equipped with a white reflective umbrella light diffuser (Photon Europe, 109 cm diameter) mounted onto a 175 cm high light stand tilted 10° downwards toward the booth. Correct and uniform exposure across the entire scene was checked before each session with a digital light meter (Sekonic L-308S). Colour calibration was performed using X-Rite Color Checker Passport colour targets and a white balance patch photographed at the beginning of each session. For further details of the photo acquisition procedure, see Třebický et al.[65].

Participants were photographed wearing provided white T-shirts and without any adornments or glasses. They had varying amount of facial hair ranging from clean-shaven to a full beard (in two participants), but most targets had a comparable style of short stubble. Participants were seated on a barstool 0.5 m from a plain white background. They were asked to sit straight with hands hanging freely alongside their bodies, look directly into the camera, and adopt a neutral expression. The camera (a sensor plane, marked φ) was positioned 125 cm from the participant and its height adjusted individually for each target to centre his head in the middle of the frame [distance between the camera and the participant was checked with a digital laser rangefinder (Bosch PLR 15)]. This setting of camera distance, focal length, and sensor size yielded a 35 × 53 cm field of view (23.85° angle of view).

Post-processing of photographs. Image processing was carried out in Adobe Lightroom Classic CC (version 2017) and Adobe Photoshop CC 2015. We converted the images into DNG raw files and created DNG colour calibration profiles (using the X-Rite Color Checker Passport Lightroom plugin). Then we applied the profiles to all photographs. The calibrated images were exported into 16-bit Adobe RGB TIFF files in their actual size (35 × 53 cm) with a 168 PPI resolution. We manually checked the exposure (using the eye-drop tool on the background above the participants' heads) and corrected the exposition on 85% value of every channel in the RGB colour space if necessary. Horizontal and vertical position of each participant in the image was adjusted using the Lightroom Transform tool (target's head was positioned into the centre of the frame with pupils on a horizontal line). Then we batch-cropped to fit the heads on 27″ monitors in 1:1 size.

In the next step, we removed any possible disturbing creases or shadows in the background. Finally, we converted the photographs into an sRGB colour space and exported them into an 8-bit JPEG format (2101 × 3031 resolution, 168 PPI, sRGB) for the rating.

Measurements of facial skin colour. *In vivo measurements with a spectrophotometer.* Facial skin colour was measured in vivo with a spectrophotometer Ocean Optics Flame-S with optical resolution of 2 nm using a standard D65 illuminant. Integrating Sphere ISP-R was used to spatially integrate the radiant flux in scatter transmission and diffuse reflectance sample measurements. The spectrophotometer was calibrated using the WS-1 Diffuse Reflectance Standard. All measurements were taken on three patches of targets' faces (forehead, left and right cheek) and expressed also in CIE L*a*b* colour space[67].

Facial photographs. We have also measured facial skin colour from the calibrated pre-vaccination facial photographs using ImageJ software (v 1.51) and Color Transformer 2 MatLab package. We measured the skin colour in CIE L*a*b* colour space and recorded the values for facial redness (a*), yellowness (b*), and lightness (L*) in three places of the face (forehead, right and left cheek)[68]. We measured the largest available area per stimulus while avoiding freckles, blemishes, and hair whenever possible. Facial skin colour values obtained from the spectrophotometer and from the facial photographs in our sample correlated positively (right cheek L* $\rho = 0.314$, left cheek L* $\rho = 0.271$, forehead L* $\rho = 0.458$; right cheek a* $\rho = 0.271$, left cheek a* $\rho = 0.187$, forehead a* $\rho = 0.442$; right cheek b* $\rho = 0.685$, left cheek b* $\rho = 0.496$, forehead b* $\rho = 0.250$). To facilitate a comparison with previous studies, we decided to use in further analyses in the main text facial skin colour measurements from the photographs. The results of analyses using spectrophotometer can be found in the Supplementary Materials—Tables S1, S2, S3, S4, S5, S6, S7, S8 and S9.

Skin patches. We cropped skin patches from the obtained facial photographs in Adobe Photoshop CC 2015. The area of skin patches (89 × 89px) and location from which they were acquired (left cheek and forehead) were standardised while making sure that the resulting patch did not include any facial features (eyes, nose), hair, or birthmarks. The resulting skin patches (left cheek N = 21; forehead N = 18, in three instances the hair was covering the foreheads and we were unable to find any suitable patch) were enlarged by 300% for subsequent presentation (as per Jones et al.[69]).

A sample of portrait with outlined skin patch can be found in Fig. 1.

Raters. Raters were recruited via social media sites (Facebook), oral invitations, and posters in university halls of the Faculty of Science, Faculty of Humanities, and the Faculty of Physical Education and Sport (all Charles University, Prague, Czechia). In Study 1, facial photographs were rated by 88 females aged 18–40 (mean = 22.87 ys; SD = 2.85) during Q1 2018. The raters received a reimbursement of 200 CZK (app. €8) as a compensation for their participation in the whole project (which also included ratings of voice recordings and body odour).

In Study 2, the obtained photographs and skin patches were rated by 62 females aged 18–40 (mean = 22.6 ys; SD = 3.42) during Q1 2019. The raters received a reimbursement of 50 CZK (app. €2) as a compensation for their time.

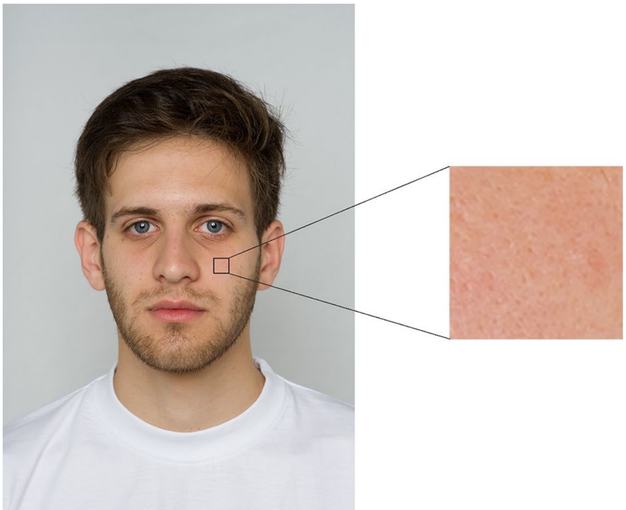

Figure 1. An example of acquired facial photograph with an outlined skin patch on the left and the resulting skin patch on the right (informed consent was obtained to publish the image in an online open-access publication).

In Study 3, the photographs were rated by 66 females aged 18–40 (mean = 23 ys; SD = 4.71) during Q4 2019. They received a reimbursement of 150 CZK (app. €6) as a compensation for their participation in a larger rating session unrelated to the current investigation.

None of the raters in the three studies used hormonal contraception.

The rating procedure. Study 1 was part of a larger project that also included the rating of voice recordings and body odour samples[53]. Exposure to a higher number of odour samples increases the risk of olfactory adaptation and can therefore affect rating, which is why the rating sessions were conducted on two separate days to accommodate a larger number of raters. From the total of 88 raters, 43 took part on the first day and 45 on the second day, which corresponds to the number of raters per photograph (depending on which day the photograph was presented). Randomly selected half of pre-vaccination samples was presented on the first rating day and half of post-vaccination photographs was presented on day two and vice versa. All raters were assessing photographs only once within a single day.

Study 2 was carried out using same rating procedure to eliminate any possible effects of a different data collection design. Of the 62 raters, 32 rated the first half of the randomly selected stimuli and 30 the second half of the stimuli. For Study 3, the procedure was identical and of the 66 raters, 31 took part on the first data collection day, 35 on the second day.

All rating (Study 1–3) took place in the Human Ethology perception lab under standardised conditions across all raters and rating days (closed window blinds, with artificial lighting to eliminate any changes in ambient lighting). The rating was conducted using Qualtrics survey suite (Qualtrics, Provo, UT) on two desktop computers of identical configuration with colour and brightness calibrated (by X-Rite i1Display Pro probe) LCD monitors (27″ Dell U2718Q UltraSharp IPS; 3840 × 2160 @ 168 DPI, 99% sRGB colour space coverage) turned to a vertical position to accommodate life-sized facial images.

The raters were seated 115 cm from the screen with eyes at a height of 125 cm (measured from the floor to the outer corner of the eye). This is a height and distance comparable to that from which the portrait photographs were taken, whereby raters were positioned into the same centre of projection and eye level. This setup approximates the common interpersonal distance[65,70]. Photographs were presented in randomised order.

In Study 1, all facial photographs (N = 21) were rated for attractiveness on a 7-point verbally anchored scale. In Study 2, participants rated portrait photographs (N = 21) on a 7-point verbally anchored scale for attractiveness again to check the robustness of acquired ratings. They also rated skin patches from left cheek (N = 21) and forehead (N = 18) on a 7-point verbally anchored scale regarding their healthiness. Due to a low number of forehead patches (hair in the images), we use only cheek patches in analysis below. In Study 3, portrait photographs (N = 21) were rated on a 7-point verbally anchored scale regarding healthiness.

After rendering their rating assessments (Study 1–3), raters completed a questionnaire about their basic demographics (age, education, occupation, etc.).

Data analyses. To determine the consistency of raters' assessments, we performed an intra-class correlation (ICC) analysis for each group rating the same set of samples using IBM SPPSS Statistics (v 23). All remaining statistical analyses were performed in jamovi (v 1.6.15).

To explore relationships between variables, ratings of facial attractiveness from Study 1 and 2, and facial healthiness from Study 3, we used Spearman's rank correlation coefficient because the data deviated from normal distribution. We set $\rho \geq 0.8$ as a value at which we would consider the two variables highly correlated. In such case, only one of the variables would be used for subsequent analyses[71]. Further, we used the Spearman's rank correlation to test the association between levels of antibodies and targets' age.

We used a one-way analysis of variance (ANOVA) with Tukey post-hoc test to test for differences and Spearman's rank correlation coefficient for strength of associations between separate colour measurements from the right and left cheek and the forehead.

To examine the relationship between perceived facial attractiveness (Study 1), perceived skin patch healthiness (Study 2), perceived facial healthiness (Study 3), and differences in antibody levels (pre-vaccination subtracted from 30 days post-vaccination), we specified three separate linear mixed-effects models (LMMs) using the GAMLj module in jamovi. The rated characteristics (facial attractiveness, healthiness, and skin patch healthiness) were entered as dependent variables, while differences in antibodies against hepatitis A (Anti-Hav) and meningococcus (Anti-Mnk) were entered as predictors. To control for variability in targets and raters, we entered the targets' and raters' IDs as random effects (example of the model entry: Facial Attractiveness ~ 1 + State/antibody levels/ + (1|ID_rater) + (1|ID_donor)). We employed analogous models to assess the relationships between the rated characteristics (facial attractiveness and healthiness), steroid hormones levels, and the percentage of adipose tissue, and to assess the relationship between the rated characteristics (facial attractiveness, healthiness, and skin patch healthiness) and forehead and cheek lightness, redness, and yellowness. To explore a possible relationship between targets' age and perceived facial attractiveness and healthiness, we ran analogous separate linear mixed-effects models, with the rated characteristic entered as a dependent variable and age as the predictor.

To test the association between differences in antibody levels (pre-vaccination and 30 days post-vaccination), basal levels of steroid hormones (testosterone and cortisol), and the percentage of adipose tissue, we employed general linear models (GLM) using the GAMLj jamovi module. In both models, we entered specific antibodies (Anti-HAV or Anti-Mnk) as dependent variables and steroid hormones and percentage of adipose tissue as predictors (e.g., Anti-HAV ~ 1 + basal cortisol + basal testosterone + adipose tissue (%)). Analogous tests were carried out to investigate the relationship between antibody levels (pre- and 30 days post-vaccination) and forehead and cheek lightness, redness, and yellowness. For information about model residuals, see Supplementary Materials S1.

We performed a simulation-based power analysis for each fixed-effect predictor in our LMMs[72] to estimate observed power using the SimR package[73] in R (for a discussion of limits of observed power, see Lakens[74]). Further, based on simulated data (gradually increasing the sample size to 100), we plotted Power curves showing the sensitivity to detect observed effects with $\alpha = 0.05$. The results of observed power, Power curve plots, and the R script are available in the Supplementary Materials S2, S3.

Results

Descriptive statistics for targets' basic demographic data, rated characteristics, differences between pre- and 30 days post-vaccination antibody levels, steroid hormone levels, the percentage of adipose tissue, and colour measurements are presented in Table 1. For detailed information, see Table S10 in Supplementary Materials.

We found high level of agreement between raters in all rated characteristics (ICC above 0.864). For further details, see Table S11 in Supplementary Materials.

Relationships between variables.

Ratings of facial attractiveness collected in Study 1 and 2 were strongly positively and statistically significantly correlated ($\rho = 0.937$, $p < 0.001$). In all subsequent analyses, we therefore use attractiveness ratings from Study 1.

Ratings of perceived facial attractiveness (Study 1) and perceived healthiness (Study 3) were also positively and statistically significantly correlated ($\rho = 0.706$, $p < 0.001$). The value of ρ did not, however, reach the pre-set level of 0.8, and we therefore analyse perceived facial attractiveness and healthiness separately.

Linear mixed-effects model testing the relationship between targets' age and perceived facial attractiveness ($R^2_C = 0.523$, $R^2_M = 0.030$) did not show a statistically significant association ($\beta = -0.063$ [-0.154, 0.028], $p = 0.193$). The relationship between perceived facial healthiness and targets' age ($R^2_C = 0.474$, $R^2_M = 0.035$) was likewise not statistically significant ($\beta = -0.069$ [-0.155, 0.016], $p = 0.130$). Further, we found no statistically significant relationship between antibody levels and targets' age ($\rho_{Anti\text{-}HAV} = -0.130$, $p = 0.573$; $\rho_{Anti\text{-}Mnk} = -0.078$, $p = 0.738$). In subsequent analyses, we therefore did not control for age.

Left and right cheek measures of skin lightness ($\rho = 0.801$, $p < 0.001$), redness ($\rho = 0.861$, < 0.001), and yellowness ($\rho = 0.925$, $p < 0.001$) were strongly positively and statistically significantly associated. We thus continue to use only L* a* b* measures from the left cheek in further analyses because we presented the left cheek patches to participants in Study 2 for patch healthiness ratings.

Skin lightness ($\rho = 0.444$, $p = 0.044$), redness ($\rho = 0.544$, $p = 0.011$), and yellowness ($\rho = 0.689$, $p < 0.001$) from the left cheek and forehead were also positively and statistically significantly correlated. The ρs did not, however, reach the predefined level (0.8) and we therefore use the left cheek and forehead measures in further analyses separately. For further details, see Table S12 in Supplementary Materials.

In our targets, skin on the forehead was statistically significantly lighter (L*) and statistically significantly less red (a*) and less yellow (b*) than skin on either cheek (for skin yellowness (b*), there was a statistically significant result for forehead and left cheek only). The two cheeks did not differ significantly in either L*, a*, or b* measures (see Fig. 2 and Tables S13–S15 in Supplemental Materials).

Study 1: association between perceived facial attractiveness, antibody levels, colouration, and immunomodulatory factors.

Linear mixed-effects models show that perceived facial attractiveness ($R^2_C = 0.532$, $R^2_M = 0.024$) was not predicted by levels of specific antibodies. For details, see Table 2.

Parameter name	Mean	SD	Range
Age (ys)	26.19	4.62	20–35
Height (cm)	181	6.74	169–198
Weight (kg)	78.9	14.8	58.5–130
Facial attractiveness S1	3.08	0.978	1.37–4.63*
Facial attractiveness S2	3.18	0.772	1.75–4.38*
Cheek patch healthiness S2	3.75	0.803	2.69–5.4*
Facial healthiness S3	4.38	0.932	2.23–5.91*
Anti-HAV antibodies (arb. U.)	−1.07	1.01	−2.11–1.55
Anti-Mnk antibodies (IU/I)	14.6	17.4	0.14–56.4
Basal testosterone (ug/l)	4.33	1.23	2.25–7.1
Basal cortisol (nmol/l)	471	91.4	282–662
Adipose tissue (%)	17.5	6.86	5.00–32.8
Left cheek lightness	67.9	2.83	63.7–74.2
Right cheek lightness	69	2.45	65.8–76.3
Forehead lightness	74.1	2.9	66.4–80.1
Left cheek redness	12.7	1.75	9.41–15.8
Right cheek redness	12.5	1.79	9.78–15.8
Forehead redness	10.2	1.77	6.54–14.7
Left cheek yellowness	18.2	2.54	14.2–23.7
Right cheek yellowness	17.3	2.16	14.5–22.1
Forehead yellowness	16.2	2.47	12.5–20.8

Table 1. Descriptive statistics for target's age, height and weight, ratings of facial photographs and skin patches before vaccination, specific antibodies (difference between states 30 days after and before vaccination), testosterone and cortisol basal levels, the amount of adipose tissue, and facial skin colour (L*a*b* for cheeks and forehead) (N = 21). Mean (SD) rating for facial photographs and skin patches was calculated as the mean from aggregated ratings for each target. Values denoted by * show the mean minimum and mean maximum ratings of photographs.

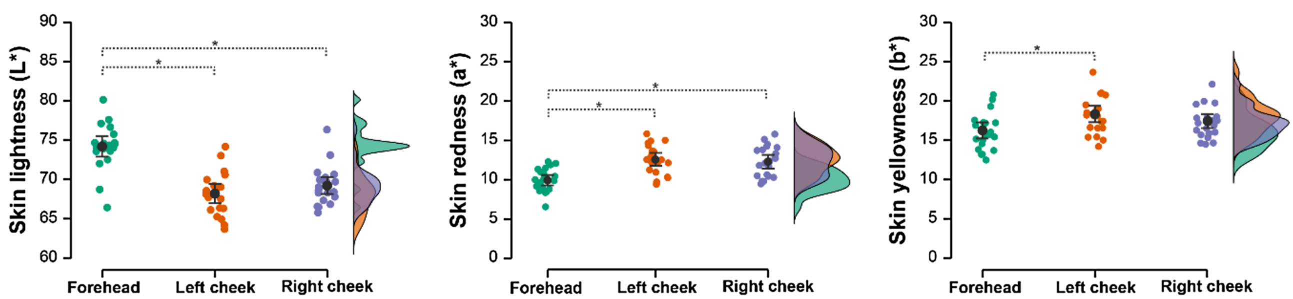

Figure 2. Differences in skin colour (CIE L*a*b*) measured from right and left cheek and the forehead. Black dots represent mean values, error bars show their 95% confidence intervals. Coloured points represent individual data points, while density plots show their distribution. Statistically significant differences are marked by asterisk.

Neither redness, yellowness, nor lightness of the forehead or left cheek predicted elevations in any of the specific antibodies (Anti-HAV: $R^2 = 0.447$, $R^2_{adj} = -0.210$; Anti-Mnk: $R^2 = 0.350$, $R^2_{adj} = 0.071$). For detailed results, see Table 3.

Running a linear mixed-effects model, we found that neither the levels of cortisol or testosterone, nor the percentage of adipose tissue predicted perceived facial attractiveness ($R^2_C = 0.534$, $R^2_M = 0.099$). For details, see Table 4.

In a GLM analysis, neither the levels of testosterone or cortisol, nor the percentage of adipose tissue predicted elevations in any of the specific antibodies Anti-HAV ($R^2 = 0.084$, $R^2_{adj} = -0.078$) and Anti-Mnk ($R^2 = 0.186$, $R^2_{adj} = 0.042$). For detailed results, see Table 5.

In a linear mixed-effects model testing the influence of skin colour on perceived facial attractiveness ($R^2_C = 0.540$, $R^2_M = 0.190$), forehead redness was the only statistically significant predictor with a negative slope ($\beta = -0.490$ [-0.780, -0.201]); see Table 6.

Characteristic	Predictors	F	β	95% CI (LL, UL)	df	t	SE	*p*
Facial attractiveness	Anti-HAV	0.955	0.217	− 0.218, 0.651	18	0.977	0.222	0.341
	Anti-Mnk	0.568	0.010	− 0.016, 0.035	18.2	0.753	0.013	0.461
Facial healthiness	Anti-HAV	0.140	− 0.080	− 0.501, 0.340	18.2	− 0.375	0.215	0.712
	Anti-Mnk	0.755	0.011	− 0.014, 0.035	18.5	0.869	0.013	0.396
Cheek patch healthiness	Anti-HAV	0.244	− 0.089	− 0.443, 0.265	18.4	− 0.494	0.181	0.627
	Anti-Mnk	1.593	0.013	− 0.007, 0.034	18.8	1.262	0.011	0.222

Table 2. Relationship between reactivity of the immune system and perceived facial characteristics. Attractiveness ratings: for target ID, VRC = 0.960, SD = 0.980, ICC = 0.429; for rater ID, VRC = 0.429, SD = 0.655, ICC = 0.251. Relationship between perceived healthiness and reactivity of the immune system. Facial healthiness ratings: for target ID, VRC = 0.876, SD = 0.936, ICC = 0.374; for rater ID, VRC = 0.458, SD = 0.677, ICC = 0.238. Relationship between perceived cheek patch healthiness and reactivity of the immune system. Cheek patch healthiness ratings: for target ID, VRC = 0.608, SD = 0.780, ICC = 0.324; for rater ID, VRC = 0.487, SD = 0.698, ICC = 0.278. *Anti-HAV* antibodies against hepatitis A, *Anti-Mnk* antibodies against meningococcus, *VRC* variance of random components.

Characteristic	Predictors	F	β	95% CI (LL, UL)	df	t	SE	*p*
Anti-HAV	Left cheek lightness	1.157	0.372	− 0.132, 0.396	14	1.075	0.123	0.300
	Forehead lightness	0.022	0.063	− 0.296, 0.340	14	0.147	0.148	0.885
	Left cheek redness	3.197	0.542	− 0.062, 0.686	14	1.788	0.175	0.095
	Forehead redness	1.060	− 0.314	− 0.552, 0.194	14	− 1.029	0.174	0.321
	Left cheek yellowness	3.439	0.936	− 0.058, 0.800	14	1.854	0.200	0.085
	Forehead yellowness	0.040	− 0.116	− 0.556, 0.462	14	− 0.200	0.237	0.845
Anti-Mnk	Left cheek lightness	0.334	− 0.216	− 6.26, 3.60	14	− 0.578	2.30	0.573
	Forehead lightness	0.555	0.345	− 3.88, 8.01	14	0.745	2.77	0.468
	Left cheek redness	0.376	0.202	− 5, 9	14	0.613	3.26	0.550
	Forehead redness	2.283	− 0.500	− 11.89, 2.06	14	− 1.511	3.25	0.153
	Left cheek yellowness	0.284	− 0.292	− 10.02, 6.03	14	− 0.533	3.74	0.602
	Forehead yellowness	0.115	0.214	− 8.01, 11.02	14	0.339	4.44	0.740

Table 3. Relationship between reactivity of the immune system and forehead and cheek lightness, redness, and yellowness. *Anti-HAV* antibodies against hepatitis A, *Anti-Mnk* antibodies against meningococcus. β represents a standardized β estimate.

Characteristic	Predictors	F	β	95% CI (LL, UL)	df	t	SE	*p*
Facial attractiveness	Cortisol	1.445	− 0.003	− 0.008, 0.002	17.1	− 1.202	0.002	0.246
	Testosterone	0.136	0.085	− 0.366, 0.536	17	0.368	0.230	0.717
	Adipose tissue	3.023	− 0.075	− 0.160, 0.010	17	− 1.739	0.043	0.100
Facial healthiness	Cortisol	5.265	− 0.005	− 0.010, − 0.001	17.3	− 2.295	0.002	0.035
	Testosterone	1.019	0.223	− 0.210, 0.657	17	1.010	0.221	0.327
	Adipose tissue	0.348	− 0.025	− 0.106, 0.057	17.1	− 0.590	0.042	0.563

Table 4. Relationship between perceived facial attractiveness and healthiness, cortisol, testosterone levels, and adipose tissue. Attractiveness ratings: for target ID, VRC = 0.763, SD = 0.873, ICC = 0.374; for rater ID, VRC = 0.429, SD = 0.655, ICC = 0.251. Relationship between facial healthiness and cortisol, testosterone levels, and percentage of adipose tissue. Facial healthiness ratings: for target ID, VRC = 0.690, SD = 0.831, ICC = 0.320; for rater ID, VRC = 0.458, SD = 0.676, ICC = 0.238. *VRC* variance of random components.

Study 2: association between perceived cheek patch healthiness and colouration. A linear mixed-effects model shows that perceived cheek patch healthiness ($R^2_C = 0.478$, $R^2_M = 0.025$) was not predicted by levels of specific antibodies (Table 2). In a linear mixed-effects model testing the influence of skin colour on perceived cheek patch healthiness ($R^2_C = 0.471$, $R^2_M = 0.164$), cheek redness negatively predicted perceived cheek patch healthiness. For detailed information, see Table 6.

Characteristic	Predictors	F	β	95% CI (LL, UL)	df	t	SE	*p*
Anti-HAV	Cortisol	1.101	0.270	−0.003, 0.009	17	1.049	0.003	0.309
	Testosterone	0.170	−0.136	−0.682, 0.458	17	−0.413	0.270	0.685
	Adipose tissue	0.100	−0.109	−0.123, 0.091	17	−0.317	0.051	0.755
Anti-Mnk	Cortisol	0.433	−0.160	−0.128, 0.067	17	−0.658	0.046	0.520
	Testosterone	3.158	0.551	−1.461, 17.07	17	1.777	4.392	0.093
	Adipose tissue	1.928	0.452	−0.594, 2.883	17	1.389	0.824	0.183

Table 5. Relationship between reactivity of the immune system, steroid hormones levels, and adipose tissue. *Anti-HAV* antibodies against hepatitis A, *Anti-Mnk* antibodies against meningococcus. β represents a standardized β estimate.

Characteristic	Predictors	F	β	95% CI (LL, UL)	df	t	SE	*p*
Facial attractiveness	Left cheek lightness	0.013	0.012	−0.197, 0.222	15.5	0.114	0.107	0.911
	Forehead lightness	0.496	−0.090	−0.340, 0.160	14.8	−0.704	0.127	0.492
	Left cheek redness	0.352	0.090	−0.206, 0.386	15.2	0.593	0.151	0.562
	Forehead redness	11.038	−0.490	−0.780, −0.201	14.1	−3.322	0.148	0.005
	Left cheek yellowness	1.865	0.232	− 0.101, 0.567	14.3	1.366	0.171	0.193
	Forehead yellowness	0.159	−0.081	−0.476, 0.315	14.2	−0.399	0.202	0.696
Facial healthiness	Left cheek lightness	3.895	0.188	0.001, 0.375	14.2	1.974	0.095	0.068
	Forehead lightness	2.671	−0.188	−0.414, 0.038	14.2	−1.634	0.115	0.124
	Left cheek redness	0.065	0.034	−0.229, 0.298	13.9	0.254	0.135	0.803
	Forehead redness	7.023	−0.357	−0.620, −0.093	14.1	−2.650	0.135	0.019
	Left cheek yellowness	0.867	0.144	−0.159, 0.447	14	0.931	0.155	0.368
	Forehead yellowness	0.182	−0.078	−0.436, 0.280	13.9	−0.427	0.183	0.676
Cheek patch healthiness	Left cheek lightness	3.035	0.112	−0.014, 0.238	17.1	1.742	0.064	0.099
	Left cheek redness	7.313	−0.240	−0.414, −0.066	16.5	−2.704	0.089	0.015
	Left cheek yellowness	0.013	0.007	−0.110, 0.123	16.6	0.113	0.059	0.911

Table 6. Relationship between perceived characteristics and facial colouration. Facial attractiveness ratings: for target ID, VRC = 0.545, SD = 0.739, ICC = 0.299; for rater ID, VRC = 0.429, SD = 0.655, ICC = 0.251. Facial healthiness ratings: for target ID, VRC = 0.433, SD = 0.658, ICC = 0.228; for rater ID, VRC = 0.458, SD = 0.677, ICC = 0.238. Cheek patch healthiness ratings: for target ID, VRC = 0.244, SD = 0.493, ICC = 0.161; for rater ID, VRC = 0.490, SD = 0.7, ICC = 0.279. *VRC* variance of random components.

Study 3: association between perceived facial healthiness, antibody levels, colouration, and immunomodulatory factors. A linear mixed-effects model shows that perceived facial healthiness ($R^2_C = 0.484$, $R^2_M = 0.015$) was not predicted by levels of specific antibodies (for details, see Table 2). In a separate linear mixed-effects model testing the influence of skin colour on perceived facial healthiness ($R^2_C = 0.491$, $R^2_M = 0.182$), forehead redness negatively predicted (β = −0.357 [−0.620, −0.093]) perceived facial healthiness (see Table 6), though we stress out the effect's 95% CIs span from substantially negative (LL = −0.620) to negligible ones (UL = −0.093).

A linear mixed-effects model ($R^2_C = 0.487$, $R^2_M = 0.086$) testing the association between cortisol, testosterone, adipose tissue percentage, and facial healthiness shows that only cortisol levels marginally negatively predicted (β = −0.005 [−0.010, −0.001]) perceived facial healthiness. For details, see Table 4.

Discussion

The main aim of all three studies was to test for possible associations between immune system reactivity (an organism's ability to effectively respond to an antigen) and perceived attractiveness and healthiness. We found no statistically significant associations between experimentally elicited levels of antibodies against hepatitis A (Anti-HAV) or meningococcus (Anti-Mnk) and perceived facial attractiveness, healthiness, or healthiness of skin patches. Moreover, we observed no statistically significant associations between the levels of antibodies and testosterone, cortisol, or adipose tissue, which are all variables often associated with immune function. Adipose tissue and testosterone and cortisol levels also showed no connection with perceived facial attractiveness. Notably, we found a small negative effect of cortisol levels on perceived facial healthiness. Further, we found that higher forehead redness was perceived as less attractive and healthy when individuals assessed portrait photographs, and for cheek patches, higher cheek redness was perceived as less healthy. No systematic relationship was found between measures of facial skin colouration and Anti-HAV and Anti-Mnk antibodies.

We examined possible relationships between immunoreactivity and facial attractiveness and healthiness because it has often been claimed that attractive traits are related to underlying qualities of individuals. Previous studies indeed reported a positive link between male facial attractiveness and either cytokine levels after stimulation with LPS ($r = 0.291$, $N = 41$)[7] or elevated immune system response to vaccination against hepatitis B ($\beta = 0.5$, $N = 74$)[9]. Although the 95% confidence intervals of our results regarding the association between Anti-HAV, Anti-Mnk, and perceived facial attractiveness ($\beta_{anti\text{-}HAV} = 0.217$, [− 0.218, 0.651]; $\beta_{anti\text{-}MNK} = 0.01$, [0.016, 0.035]; $N = 21$) do partially overlap with results of some studies that found significant relation between perceived facial attractiveness and hepatitis B antibodies levels after vaccination[9], our results are more in line with the studies by Skrinda et al.[11] and Rantala et al.[10] who found no support for significant associations between hepatitis B antibody levels after vaccination and perceived attractiveness in men ($\beta = -0.21$, $N = 60$) and women ($r = -0.006$, $N = 52$), respectively. Overall, as noted at the outset, empirical evidence regarding an association between immunocompetence and facial attractiveness remains equivocal[75].

The strength of our study lies in using vaccines against both viral (hepatitis A and B) and bacterial (meningococcus) diseases. This way, we aimed to stimulate a wider array of immune system components because, for example, the advantage of heterozygotic individuals is the greatest when they fight against multiple pathogens at once[76]. Moreover, we inspected both immunoreactivity and facial colouration. Unlike some previous studies[9,50], we excluded hepatitis B antibodies from our analyses because several participants showed high levels of the relevant antibodies already in the baseline measurement, while others did not react to the vaccine.

In general, the use of vaccination to stimulate immunoreactivity has some limitations. For the purpose of this study, we treated a higher level of antibodies as a proxy to higher disease resistance. This is, however, something of a simplification because higher immunoreactivity is not always adaptive[77]. Excessively strong (hypersensitivity) or inappropriate (e.g., autoimmune) immunity response is not beneficial and can ultimately negatively affect individual fitness. Moreover, by focusing solely on antibody levels, one can only arrive at generalised and limited information about the function of the immune system. Investigation of differences of the immune response in its humoral and cellular components and of the trade-offs between them might provide a more nuanced insight.

Recent studies employed several methods of measuring the functioning of the immune system and arrived at rather diverse results, making our null results no exception. Foo et al.[78] focused on innate immunity and measured salivary immune function (antibacterial capacity against *Escherichia coli* and lysosome activity against *Micrococcus lysodekticus*) alongside oxidative stress and semen quality. Using principal component analyses, they obtained two factors: PC1—bacterial-killing capacity and overall bacterial immunity and PC2—bacterial suppression capacity and lysozyme activity. Contrary to expectations, no connection was found between the selected physiological measures of immune function, attractiveness ($r_{PC1} = -0.16$, $r_{PC2} = 0.04$, $N = 98$) and number of sexual partners ($r_{PC1} = -0.07$, $r_{PC2} = -0.10$, $N = 97$)[78]. Phalane et al.[7], on the other hand, found a positive relationship between cytokine levels (after stimulation of the immune system with LPS) ($r = 0.291$, $N = 41$) and male facial attractiveness but not the CRP ($r = -0.085$, $N = 41$)[7]. Cai et al.[79] employed as a marker of immune function salivary immunoglobulin A (IgA), which acts as a defence against microbial invasion. They found no connection between IgA and female facial attractiveness ($\rho = -0.051$, $N = 221$)[79].

It has been proposed that facial skin provides information about the functioning of the immune system and about health[16,69,80]. It has been demonstrated that people can assess health and attractiveness even from limited information such as skin patch and their ratings correspond to their ratings of the whole face[15,69]. In our study, we therefore used cheek skin patches to limit possible effects of confounding factors (e.g., face shape). We also investigated relationships between perceived healthiness of the skin patch and direct measures of immune system function. And yet, we found no associations between perceived skin healthiness and levels of specific antibodies.

Previous studies reported that testosterone and cortisol have an effect on both the functioning of the immune system and perceived facial attractiveness and healthiness, and might thus work as mediators between the functioning of the immune system and perceived facial characteristics. According to the hypothesis of immunocompetence handicap, androgens exert immunosuppressive effects and only high-quality individuals (including their immunity) can produce and maintain a high level of testosterone and afford the physiological costs of lowered immunosuppression[40]. Although the results of some studies do support the hypothesis of immunosuppressive effects of sex hormones[38,81], the overall pattern in literature is rather mixed (see, e.g., a metanalysis[41]). It has been suggested that glucocorticoids contribute to this complex picture because they modulate immune system response as well as the expression of secondary sexual characteristics, and they may interact with testosterone[42,43,45]. In our study, we found no significant effects of either testosterone or cortisol on antibody levels. This finding is consistent with the results of Nowak et al.[82], who found no influence of testosterone on the effectiveness of immune system using the influenza vaccine. In contrast, though, in vitro studies did find an immunosuppressive effect of testosterone on a spontaneous production of IgG in mononuclear cells of human peripheral blood[83,84]. Rantala et al.[9], however, showed that the immune system's reactivity was higher in males with higher testosterone levels who simultaneously exhibited lower cortisol levels and, moreover, these males were perceived as more attractive by women. In our study, we found none of the expected associations between testosterone and the rated characteristics. We found only a weak negative association between cortisol and perceived healthiness, but not attractiveness. Interestingly, another study showed a negative association between attractiveness (but not healthiness) and cortisol levels[45].

Additionally, we found that the two scales of facial attractiveness and healthiness are positively correlated but the magnitude of this association is not strong enough to treat the two as interchangeable. It is thus possible that facial attractiveness and healthiness stand for two separate perceptual qualities. This idea finds further support both in the negative association between cortisol levels and perceived facial healthiness reported in our study and in the negative association between cortisol levels and perceived attractiveness in a study by Moore et al.[45]. This suggests that one should exercise caution when selecting specific characteristics to be rated for individual studies.

It has been reported that higher adiposity contributes to reduced immunocompetence, and possibly impaired immune function accompanied by changes in leukocyte counts, lower antibody production, as well as worse wound healing and higher risk of infections[47,48] Moreover, the faces of obese and overweight individuals are perceived as less attractive[5,51]. Adiposity thus seems to underlie the relationship between immune response and attractiveness[50]. In our study, we did not find any significant relationship between perceived facial attractiveness or healthiness and antibody levels or body fat percentage. One possible explanation might be that participants in our sample had a generally lower body fat percentage (mean = 17.5%): only two participants fell in the obese category with body fat percentage over 25% (threshold recommended by the American Council on Exercise). Our sample, where variability of body fat percentage was relatively low, may have been thus ill-suited to detecting the negative effect of increased adiposity. On the other hand, other studies detected a negative effect of higher weight (expressed by BMI) on immune function even within the range of average body weight variation[85].

A number of previous studies reported associations between skin colour, facial appearance, and immune response. South African men with a higher cytokine response to stimulation (induced by LPS) had yellower, more 'carotenoid' skin colour[7]. Furthermore, yellower skin was preferred alongside lighter skin, but it is well possible that this preference for lighter skin is due to the yellow carotenoid colouration being more visible in lighter skin hues[7]. In our study, however, we found no statistically significant associations between skin yellowness and perceived characteristics. A number of other studies (e.g., Stephen et al.[17,24]) employed manipulation of skin colour in photographs, while we used natural portrait images. This may have resulted in a lower variability in our sample, thus potentially reducing the likelihood of observing the effect. Still, we found that both perceived facial attractiveness and healthiness were negatively predicted by higher forehead redness and cheek skin healthiness was negatively predicted by higher cheek redness. Although higher redness has been previously linked to higher perceived attractiveness and health[16,17], the relationship need not be linear: it is possible that some level of redness may affect perceived attractiveness positively, but above a certain threshold it has a negative effect on perceived attractiveness[16]. We propose that higher (forehead) redness levels might be perceptually linked to dermatoses, such as rosacea[86], acne, or other imperfections which are generally perceived as less attractive[80].

Unlike various studies which measured skin colour from both cheeks and the forehead and averaged them into one value for facial skin lightness, redness, or yellowness[7,25,87], we used facial skin colour measurements from the cheek and the forehead separately, as majority of colour measurements between those areas differed and were only moderately associated. Cheeks and forehead differ in the amount of subcutaneous fat and therefore also in blood perfusion, which might account for slight differences in colouration. Accordingly, it has been found that the variation of colour in different parts of the face matters, whereby for instance periorbital luminance, cheek redness, and overall yellowness of the face predict perceived health[88]. To some extent, though, the differences in the skin colour of various parts of the face in our sample might be also due to the methods we used for acquisition of facial photographs from which we measured the values of facial colours. Our aim was to simulate naturally occurring daylight conditions with a diffuse strobe light positioned above the participant's head pointing downwards. In this setup, though, the light source was positioned relatively close. Due to inverse-square law of loss of light over distance, it may have reflected on the forehead, causing it to appear brighter than the cheeks, and it may have produced highlights responsible for the observed colour differences between forehead and cheeks. Further, we tried to limit any potential effects of bright spots and light reflections by patting participants' foreheads with napkins and we waited for some time before taking the photographs to avoid any skin redness caused by this process. Still, some brighter areas may have appeared and caused specular highlights, thus affecting the measurements.

Limitations. Aside from the limitations discussed above, the main limitation of the present study is the small sample size of targets (although comparable to some previous studies[89]), which resulted in wide confidence intervals of the effect sizes and a low power to observe the reported effects (in most cases below 50%). Based on our Power curve analysis, even a sample size of 100 targets would not yield a higher power (e.g., ≥ 80% for most effects).

We experienced significant obstacles in participant recruitment due to the relatively strict conditions for participation (we required not being vaccinated against either of the diseases of our interest in the past 10 years) as well as anti-vaccination biases which may have discouraged some individuals from participation[90]. Moreover, some participants showed high levels of antibodies against hepatitis B despite our entry requirement of not being recently vaccinated. Some participants may thus have been unaware of a relatively recent vaccination. Moreover, some participants did not respond to the hepatitis B vaccine, a phenomenon observed in app. 10% of population[91]. Therefore, we had to exclude antibodies against hepatitis B from our analyses. Note, that although hepatitis B vaccine is commonly used in other studies, vaccination against hepatitis B was in 2001 included in the compulsory vaccination protocol in Czechia. Consequently, when selecting a vaccine, the context of its use ought to be investigated more closely and a choice of vaccines against some less common diseases may be a better option.

Previous research has also suggested that immunoreactivity and cues to various aspects of immune system functioning may differ between the sexes[9,10] and our results are based only on a male sample. Future investigations should thus include both men and women as targets and raters to better understand the complex relations between attractiveness and immunity and its role in intersexual selection.

Conclusion

We investigated the relationship between functioning of the immune system and perceived facial attractiveness, healthiness, skin patch healthiness, and potential influence of skin colour. We employed measurements of antibodies after application of two different vaccines as markers of reactivity of the immune system and recorded the levels of steroid hormones (cortisol and testosterone) as well as the percentage of adipose tissue due to their immunomodulatory properties and connection to facial attractiveness. We found no significant relationships between reactivity of the immune system and perceived facial attractiveness, healthiness, and skin patch healthiness. We did, however, observe a small negative effect of cortisol on perceived facial healthiness. Moreover, steroid hormones and adipose tissue showed no relationship to either the immune response after vaccination or skin colouration. Finally, higher forehead redness from portrait photographs was perceived as both less attractive and healthy and higher cheek redness from skin patches was perceived as less healthy. Our results thus suggest that facial attractiveness and healthiness provide a limited amount of cues to immune system functioning and perceived characteristics seem to be related only to certain hormone levels and facial colour.

Despite some limitations, we believe that our study is a valuable contribution to research on the role of visual cues in assessments of functioning of the immune systems of individuals, and that it can serve as an entry for future meta-analysis aimed at disentangling the conflicting results of various existing studies. Future studies might also investigate the activation of different components of the immune system, such as humoral and cellular immunity, and focus on acquiring larger samples.

Data availability

The data associated with this research are available at https://osf.io/4k3ud/.

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Author contributions
Conceptualization: Ž.P., D.S., J.T.F., V.T., J.H.; Data Curation: Ž.P., D.S.; Formal Analysis: Ž.P.; Funding Acquisition: Ž.P., D.S., V.T.; Investigation: Ž.P., D.S., D.St.; Methodology: Ž.P., D.S., J.T.F., V.T., D.St., K.K., J.H.; Project Administration: Ž.P., D.S.; Supervision: V.T., J.T.F., J.H.; Validation: Ž.P., D.S., J.T.F., V.T., D.St., K.K., J.H.; Visualization: Ž.P., V.T.; Writing—Original draft preparation: Ž.P., J.T.F.; Writing—Review and Editing: Ž.P., D.S., J.T.F., V.T,. J.H., D.St., K.K.

Competing interests
The authors declare no competing interests.

Additional information
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CHAPTER 7

IMMUNOACTIVATION AFFECTS PERCEIVED BODY ODOR AND FACIAL BUT NOT VOCAL ATTRACTIVENESS

Evolutionary Psychology

Immunoactivation affects perceived body odor and facial but not vocal attractiveness

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Manuscript ID	EVP-23-0046.R1
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Abstract:	Several previous studies have shown that in mammals, the health status of conspecifics can be assessed based on perceptual cues. Olfactory, visual, or acoustic cues may lead to avoidant behavior, thus reducing the risk of contagion by close contact with infected individuals. We tested whether immune system activation after immunization leads to perceptible changes in body odor and facial and vocal attractiveness in humans. We have experimentally activated the immune system of male subjects using vaccination against hepatitis A/B and meningococcus. Their body odor, facial photographs, and vocal recordings were collected before and 14 days after vaccination. Subsequently, the body odor samples, facial photographs, and vocal recordings were assessed by female raters for their attractiveness and healthiness. We have also measured skin coloration (from facial photographs and in vivo using a spectrophotometer), vocal parameters (F0, formants (F1-F4), HNR and CPPs), and C-Reactive Protein (CRP) levels as a marker of inflammation. We found an increase in perceived body odor attractiveness, a decrease in facial attractiveness and healthiness, and no change in vocal attractiveness 14 days after vaccination compared to the pre-vaccination state. Moreover, there was no change in facial coloration or vocal parameters between the pre- and post-vaccination conditions. Pre-vaccination CPR levels were negatively associated with body odor and facial attractiveness and positively associated with body odor intensity. Overall, our results suggest that perceived body odor as well as facial attractiveness may provide cues to activation of the immune response and that each modality carries different information about the individual's condition.

Immunoactivation affects perceived body odor and facial but not vocal attractiveness

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CONFLICT OF INTERESTS

The Authors declare that there is no conflict of interest.

ABSTRACT

Several previous studies have shown that in mammals, the health status of conspecifics can be assessed based on perceptual cues. Olfactory, visual, or acoustic cues may lead to avoidant behavior, thus reducing the risk of contagion by close contact with infected individuals. We tested whether immune system activation after immunization leads to perceptible changes in body odor and facial and vocal attractiveness in humans.

We have experimentally activated the immune system of male subjects using vaccination against hepatitis A/B and meningococcus. Their body odor, facial photographs, and vocal recordings were collected before and 14 days after vaccination. Subsequently, the body odor samples, facial photographs, and vocal recordings were assessed by female raters for their attractiveness and healthiness. We have also measured skin coloration (from facial photographs and *in vivo* using a spectrophotometer), vocal parameters (F0, formants (F1-F4), HNR and CPPs), and C-Reactive Protein (CRP) levels as a marker of inflammation.

We found an increase in perceived body odor attractiveness, a decrease in facial attractiveness and healthiness, and no change in vocal attractiveness 14 days after vaccination compared to the pre-vaccination state. Moreover, there was no change in facial coloration or vocal parameters between the pre- and post-vaccination conditions. Pre-vaccination CPR levels were negatively associated with body odor and facial attractiveness and positively associated with body odor intensity. Overall, our results suggest that perceived body odor as well as facial but not vocal attractiveness may provide cues to activation of the immune response and that each modality carries different information about the individual's condition.

Keywords: perception; health status; body odor; face; voice

1 INTRODUCTION

Social species are constantly threatened by infectious diseases. This is due to high population densities and various social interactions, which lead to a higher likelihood of pathogen transmission than in solitary species (Altizer et al., 2003). On the other hand, social species have also developed various avoidance mechanisms and behaviors to lower the risk of contagion. The most important part of these mechanisms is the early detection of threatening stimuli.

It has been shown that various social species, including humans, can assess the health status of conspecifics based on various perceptual cues. Hamilton and Zuk (1982) proposed the 'contagion indicator hypothesis', which states that male traits serve as a sensitive indicator of health status and pathogen resistance (Hamilton & Zuk, 1982). These traits may take the form of visual cues, such as the quality of fur or plumage (e.g., Zuk et al., 1990), or olfactory cues, such as urine, feces, or body odor (e.g., Penn & Potts, 1998). Even vocal (e.g., Lopes & König, 2016) or tactile cues (Sarabian, Ngoubangoye, & MacIntosh, 2017) may substantially contribute to the detection of sick conspecifics. While most studies in this area of research focus on mate choice, assessment of the health status of conspecifics is not restricted to this context. In fact, it can be highly relevant also to other social interactions, where its purpose is to avoid possible transmission of pathogens.

There is robust evidence showing that female mice distinguish between the odor of healthy males and those infected with various ecto- and endoparasites. In several studies, female mice showed a strong preference for the urine of control males (males injected with distilled water) compared to the urine of parasitized males (e.g., Kavaliers & Colwell, 1995; Kavaliers et al., 1997, Kavaliers et al., 2003a, Zala, Potts & Penn, 2004). Similarly, Arakawa et al. (2009, 2010) found preference for the smell of urine of healthy individuals in rats using lipopolysaccharide (LPS), a substance which activates the response of the physiological immune system and leads to behaviors characteristic of sickness, such as lack of activity, sleepiness, or reduction of grooming.

In humans, too, body odor samples from individuals infected with gonococcus *Neisseria gonorrhoeae* were rated as less pleasant and described as more putrid than samples from healthy individuals (Moshkin et al., 2012). Sarolidou et al. (2020) showed that body odor samples from individuals with naturally occurring respiratory infections were nominally rated as more intense, more disgusting, less pleasant, and less healthy than samples from the same participants when healthy. Moreover, odor samples collected from men injected with LPS were perceived as more aversive (Olsson et al., 2014).

Although studies of rodents tend to focus on olfactory cues to their health status, investigations of the preference for healthy individuals are not limited to this modality. Various visual cues, such as ornaments, coloration, or behavior, may likewise be assessed because infections can have a negative impact on them. For example, chimpanzees tend to avoid conspecifics who display motoric cues to disease (Goodall, 1986).

In humans, sight is the most studied modality in the context of detection of currently sick individuals. It has been proposed that cues to perceived facial attractiveness are positively associated with health, but existing evidence is rather equivocal (for a review, see Foo et al., 2017; Stephen & Luoto, 2021). One of the traits which influence perceived attractiveness is skin color (Fink et al., 2006), which is affected by current health status (Henderson et al., 2017). For instance, significant changes in skin color were observed even just one hour after LPS injection, and they varied between body regions: facial skin became lighter and less red, while skin on the arms became darker, less red, and less yellow (Henderson et al., 2017). Skin color changes could thus serve as a cue to acute illness, although they do not predict overall susceptibility to infectious illnesses (Cai et al., 2019). Besides skin coloration, body fat levels also affect attractiveness ratings, whereby both excessively thin and overweight individuals are rated as less attractive (Coetzee et al., 2009; Rantala et al., 2013b). These two states are not only rated as less attractive but also as associated with various health problems (e.g., Brown et al., 2009). Detection of illness is not restricted to one's cultural experience: people can detect and discriminate the faces of sick individuals as soon as just two hours after LPS-induced activation of the immune system regardless of sharing – or not – the ethnic origin with the ill subject (Arshamian et al., 2021).

Infection may also affect vocalization, which is in various species a trait that plays a substantial role in many social interactions, including mate choice. It has been proposed that acoustic cues provide honest information about the individual's quality and/or condition (Xu et al., 2013). For example, LPS-injected males of the house mouse produced a lower number of regular ultrasonic syllables (regarded as sexually attractive) and a larger number of (non-attractive) high-frequency ultrasonic syllables (Lopes & König, 2016). This suggests a decrease in the production of sexually attractive acoustic signals. In humans, vocal characteristics such as the fundamental frequency or formant position could likewise be linked to current health status. In men, more masculine voices (with relatively low fundamental frequency and low formant positions) are associated with better general health and higher salivary levels of immunoglobulin A, which is a biomarker of immune function (Arnocky et al., 2018). Although fundamental frequency negatively influenced healthiness ratings, raters could not assess the health status of male speakers from their voices alone (Albert et al., 2021). Regarding sounds connected with ongoing diseases, such as coughs and sneezes, a study had shown that although raters were unable to distinguish whether the sounds came from healthy or genuinely ill individuals, sounds rated as more disgusting were also judged as more likely to come from sick individuals (Michalak et al., 2020).

In this study, our aim was to test possible differences in the rating of body odor, facial and vocal attractiveness, and healthiness of men before and after vaccination. We used a vaccination against both a bacterial and a viral infection simultaneously to stimulate the complex upregulation of immune system. We were not interested in the specific immune responses because the mechanism of changes in body odor or facial and vocal attractiveness elicited by immunostimulation is as yet unknown. We predicted that abovementioned characteristics will be rated as less attractive and less healthy after vaccination. Moreover, we have anticipated that the levels of C-reactive protein (CRP), a widely used clinical marker of inflammation, would be higher after vaccination than before it. CRP was chosen as a marker of potential acute-phase reaction that could occur due to immunostimulation. Some previous studies have shown a negative association between perceived facial healthiness or attractiveness and CRP levels (Żelaźniewicz et al., 2020; Phalane et al., 2017), which is why we investigated associations between the rated characteristics and CRP levels in both states, i.e., both pre- and post-vaccination.

2 METHODS

This study is part of a larger project aimed at testing the association between immunoreactivity (measured by increased specific antibodies after vaccination), body odor quality (see Schwambergová et al., 2021), and facial attractiveness (see Pátková et al., 2022). The project was conducted at the Charles University (Prague, Czech Republic) from Q4 2017 to Q4 2019 in collaboration with the medical personnel of the Prevedig laboratory and Naděje Kočnarová, MD. All procedures were approved by the Institutional Review Board of the Charles University (approval no. 20/2016) and conducted in accordance with the Helsinki Declaration. The study design was preregistered prior to data analyses (https://osf.io/69zgc/).

2.1 Body odor donors: Targets

In total, 21 Czech men aged 18–40 years (mean = 26.2; SD = 4.62) provided body odor samples, facial photographs, and voice recordings. Participation requirements were good general health, non-smoking, not shaving one's armpits (Kohoutová, Rubešová & Havlíček, 2011), and not being vaccinated against hepatitis A/B or meningococcus for the past ten years (e.g., Shepard et al., 2006). Participants were informed about the goals of the study before its initiation and indicated their consent by signing an informed consent form. As compensation for their time and potential inconvenience, participants received 400 CZK (approx. €15) and the first dose of vaccines for free.

2.2 Procedure

Body odor samples, facial photographs, measurements of facial skin color, and voice recordings were collected twice: once during the night (body odor) or day (facial photographs and voice recordings) before vaccination, and the second time 14 days after vaccination, at a time when one could expect the highest antibody response (De Paula, 2012). Before vaccination, all targets completed a medical history form and their health status was assessed by a general practitioner. Afterwards, the targets were vaccinated against hepatitis A/B and meningococcus; for a detailed description of the vaccines, see below. We have collected from the targets three blood samples to assess the levels of CRP, specific antibodies, and steroid hormones. To determine the basal levels of these variables, we collected a blood sample before vaccination. The second sample was collected 14 days later, and the last one 30 days post-vaccination to assess the dynamics of changes in antibody levels after vaccination while observing the recommended interval for the second dose of the hepatitis vaccine (e.g., Galson et al., 2015). Vaccination was performed by a physician who also collected the initial blood samples. Phlebotomists collected other blood samples at the Prevedig laboratory, which also performed analyses of CRP levels. All blood samples were collected at the same time of the day (7–8 am) to minimize potential variation in steroid hormone levels due to circadian rhythms (Reinberg et al., 1978); results regarding the levels of steroid hormones and specific antibodies can be found in Schwambergová et al., 2021 and Pátková et al., 2022. For the study schedule, see Fig. 1.

Figure1

2.2.1 Vaccine characteristics

To induce an immune system response, we used the Menveo vaccine against meningococcus and the Twinrix Adult vaccine against hepatitis A/B. Menveo is applied to prevent (bacterial) meningococcal diseases caused by *Neisseria meningitis* serogroups A, C, Y, and W-135 (see prescription information: https://gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Menveo/pdf/MENVEO.PDF). The Twinrix Adult vaccine is used for immunization of adults against viral hepatitis A and B (https://id-ea.org/wp-content/uploads/2012/05/Twinrix-Package-Insert.pdf). These vaccines can be applied together and are widely used in the Czech Republic. Both were applied intramuscularly (in the deltoid muscle), each in one arm.

2.2.2 Body odor collection

For body odor sampling, each donor received a list of instructions and a package containing plain cotton pads (approx. 9 × 7 cm; DM Ebelin), 100 % cotton white T-shirt (Adler Malfini Heavy), a non-perfumed soap (Balea ultrasensitive), and surgical tape (Omnisilk 2.5 cm x 9.2 m). On the day before and on the day of sampling (i.e., for about 48 hours), donors were asked to avoid consuming aromatic foods, such as spices, blue cheese, or garlic, alcoholic beverages or other drugs, to refrain from strenuous physical activity, such as jogging or sex, and not to apply fragranced products, which may all affect the quality of the body odor (e.g., Havlicek & Lenochova, 2006; Lenochová, Roberts & Havlíček, 2009). Donors' conformity with these instructions was checked by a questionnaire (see Appendix 2 in Schwambergová et al., 2021) completed when handing over the body odor samples. On the night of sampling, donors washed their armpits using the non-perfumed soap by us and then attached the cotton pads to both armpits using the provided surgical tape. To limit contamination by extrinsic ambient odors, they wore a 100 % cotton T-shirt previously washed without any fragranced detergent as the innermost layer of clothing. They wore the cotton pads for 12 hours overnight (it has been demonstrated that this sampling duration is sufficient for body odor collection; cf. Havlíček et al., 2011). The next morning, they removed the cotton pads, placed them in zip-lock plastic bags, and returned them to the experimenters. The odor samples were immediately placed in a freezer set to -20 °C to limit any further microbial activity that could alter the quality of the collected body odor (Lenochová, Roberts & Havlíček, 2009). Samples were then kept in the freezer until the rating session.

2.2.3 Acquisition of facial photographs

Facial photographs and voice recordings together with measurements of body composition (for details see Schwambergová et al., 2021) and skin color (for details, see Pátková et al., 2022) were acquired in the Human Ethology laboratory at the Faculty of Science (Charles University).

Facial photographs were taken under standardized conditions in a purpose-built photographic booth to prevent any changes in illumination and color reflections. They were acquired using a 24-megapixel full-frame (35.9 × 24 mm CMOS sensor, a 35 mm film equivalent) DSLR camera Nikon D610, with a Nikon AF-S Nikkor 85 mm F1.8 AF-S G lens. Exposure was manually set to ISO 100 with shutter speed of 1/125 s and an aperture of F8 (Třebický et al., 2016). One studio strobe (Menik MD-400Ws) with a white reflective umbrella as a light modifier placed above the camera was used as the light source. The light was mounted onto a 175 cm high light stand and tilted 10° downwards toward the target. Correctness and uniformity of exposure and color settings were checked before each session using a digital light meter Seconic L-308DC and color calibration targets X-rite ColorChecker passport, respectively.

Each participant was seated on a barstool positioned 50 cm in front of a plain white background and instructed to remove any facial adornments and wear a white T-shirt provided by the researchers. Targets were asked to sit straight, with hands hanging freely alongside their bodies, look directly into the camera (Hehman et al., 2013; Třebický et al., 2019), and maintain a 'neutral' facial expression. Photographs were taken from a 125 cm distance, whereby the camera was placed on a tripod with height set depending on the participant's height so as to keep the face in the middle of the frame, with focus set on the right eye in the AF-S mode. The distance between the target and the camera (sensor plain marked ф) was verified with a digital laser rangefinder (Bosch PLR 15). This setting of camera distance, focal length, and sensor size gave a 35 × 53 cm field of view (23.85° viewing angle).

All facial photographs were post-processed using Adobe Lightroom Classic CC (version 2017) and Adobe Photoshop CC 2015. All facial photographs were color and exposure calibrated and then exported into 16-bit Adobe RGB TIFF files in their actual size (35 × 53 cm) with 168 PPI resolution. Vertical and horizontal position of each participant in the image was adjusted so that the target's head was in the center of the frame with both pupils on the horizontal line. For further details of the photo acquisition and post-processing procedures, see Třebický et al. (2018).

2.2.4 Measurements of facial skin color

Facial skin color was measured *in vivo* with spectrophotometer Ocean Optics (OO) Flame-S with optical resolution of 2 nm, using a standard D65 illuminant. Integrating OO Sphere ISP-R was used to spatially integrate the radiant flux to scatter transmission and diffuse reflectance sample measurements. The spectrophotometer was calibrated using the WS-1 Diffuse Reflectance Standard. All measurements were taken on three regions of the targets' faces (forehead, left and right cheek) and expressed in CIEL*a*b* color space (Hunter, 1958; Huang et al., 2018).

We have also measured facial skin color from calibrated pre-vaccination facial photographs using ImageJ software (v 1.51) and Color Transformer 2 MatLab package. Skin color was measured in the CIE L*a*b color space and values for redness (a*), yellowness (b*), and lightness (L*) (Henderson et al. 2016) were recorded in three regions of the face (forehead, right and left cheek) and on the inner side of biceps (which was not used for the further analysis). We measured the largest available area per stimulus while avoiding freckles, blemishes, and hair. Facial skin color values obtained from the spectrophotometer and from facial photographs taken before vaccination correlated positively (right cheek L* $\rho = 0.314$, left cheek L* $\rho = 0.271$, forehead L* $\rho = 0.458$; right cheek a* $\rho = 0.271$, left cheek a* $\rho = 0.187$, forehead a* $\rho = 0.442$; right cheek b* $\rho = 0.685$, left cheek b* $\rho = 0.496$, forehead b* $\rho = 0.250$) and the same applies to color measurements after vaccination (right cheek L* $\rho = 0.606$, left cheek L* $\rho = 0.502$, forehead L* $\rho = 0.368$; right cheek a* $\rho = 0.023$, left cheek a* $\rho = 0.292$, forehead a* $\rho = 0.308$; right cheek b* $\rho = 0.659$, left cheek b* $\rho = 0.729$, forehead b* $\rho = 0.699$). For better comparison with other studies, we decided to use in further analyses in the main text measurements of facial skin color based on photographs. For analyses using spectrophotometer, see Supplementary Material Table S1-S8.

2.2.5 Voice recordings

Voice recordings were obtained in an acoustically treated, purpose-built photographic booth using cardioid condenser microphone RØDE NT-1A equipped with pop-up and acoustic reflection filters (to reduce any potential disruptive sounds and echoes) and connected to a PC through an I/O audio interface Focusrite Scarlett Solo Gen2. The microphone was mounted on a tripod at the height of the participant's mouth. Voices were recorded via Audacity 2.1.3. into WAV files in 24bit/192 kHz resolution. Participants stood 40 cm from the microphone. The distance and other volume-related settings were kept constant to standardize the intensity of recordings. Participants were instructed to read aloud consonants, vowels, and a sentence ("My name is Peter and I come from Prague") in Czech from a provided sheet. For ratings and analyses, we used only the abovementioned sentence, which was used also in our other studies (e.g., Šebesta et al., 2017).

Acoustic analysis of the recorded sentences was performed with VoiceLab 1.2.0 (Feinberg, 2022; Feinberg & Cook, 2020). For extraction of all acoustic parameters, we have used VoiceLab's default setting (the Voicelab settings file and results file are downloadable from https://osf.io/4k3ud/). In further analyses in this study, we used only the cepstral peak prominence (CPP) as an objective measure of breathiness, harmonics-to-noise ratio (HNR) as an indicator of vocal aging, fundamental frequency (F0), which is related to voice pitch and formants (F1-F4) which are related to a resonance in the vocal tract.

2.3 Raters

In total, 88 Czech women aged 18–40 years (mean = 22.9; SD = 2.85) participated as stimuli raters. Only female raters were recruited, because they score on average better on different areas of olfactory perception (for a review, see Brand & Millot, 2001) and they consider body odor more important when selecting a possible partner than men do (Havlicek et al. 2008). Requirements for participation were good respiratory health and no use of hormonal contraception.

Facial photographs were rated twice (total N = 154): once for attractiveness during the session with body odor samples and voices (N = 88) (in Q1 2018) and then for healthiness during a rating session not directly related to the current study in Q4 2019. In the second session, photographs were rated by 66 females aged 18–40 years (mean = 23; SD = 4.71) with the same requirements for participation as outlined above. As compensation for their time, raters received 200 CZK (approx. €8) and 150 CZK (approx. €6) for participation in the first and second session, respectively.

2.4 The rating procedure

Rating of body odor samples took place in a well-ventilated, quiet room. The samples were presented in 500ml opaque jars with ground glass sealing lids labelled by a non-specific code. Each sample was rated for attractiveness, intensity, and healthiness on a 7-point verbally anchored scale (e.g., 1 – very unattractive, 7 – very attractive). The rating took place over two days (43 raters in Day 1, 45 in Day 2) to logistically accommodate the total number of raters. Ambient temperature was 18.2–20.7 °C (Day 1) and 18.7–20.6 °C (Day 2), with humidity at 28–31 % (Day 1) and 27–28 % (Day 2). During one rating day, raters were presented with either pre- or post-vaccination sample from any given odor donor (N = 21) and on the second day, they were presented with the odor donor's sample from the other condition (N = 21). For each day, the jars containing odor samples were randomly divided in three subsets and during rating, raters took breaks between each set to avoid sensory adaptation. Samples were presented in a randomized order to avoid systematic bias within a rating day. Raters were instructed to remove the lid (sealing the jar afterwards), sniff the sample, and write down their rating immediately after sniffing. The time spent sniffing was not restricted (for further details, see Schwambergová et al., 2021).

Rating of facial photographs took place in the Human Ethology perception lab under controlled settings, which were kept constant for all raters and rating days (closed window blinds, artificial illumination to reduce ambient lighting variations). The rating was conducted on two identical desktop computers with color and brightness calibrated (by XRite i1Display Pro probe) LCD screens (27" Dell U2718Q UltraSharp IPS; 3840 x 2160 @ 168 DPI, 99 % sRGB color space coverage) turned to a vertical position to accommodate life-sized facial pictures. The rating itself was conducted in the Qualtrics survey suite (Qualtrics, Provo, UT). Facial photographs were presented in a randomized order and rated on a 7-point verbally anchored scale separately for attractiveness and healthiness during the first and the second session, respectively. The raters were seated 115cm from the screen, with eyes at the height of 125 cm (measured from the floor to the outer corner of the eye). This setting closely emulated conditions under which the photographs were taken while simulating the usual interpersonal distance (Sorokowska et al., 2017; Třebický et al., 2018). Following the evaluation, raters were asked to fill in an anonymous questionnaire on their demographic data (e.g., place of residence, education, occupation) and olfactory abilities (e.g., self-rated olfactory abilities, allergies, recent or current common cold).

Voice recording rating sessions were conducted using a purpose-built rating experiment in PsychoPy (Peirce et al., 2011; v. 1.6) on two identical desktop computers (same as for photography rating) with Focusrite Scarlett Solo Gen 2 audio I/O interfaces and studio reference Beyerdynamic DT 770 Pro 32 Ohm over-ear closed headphones (5–35 kHz). Recordings were played from original uncompressed

WAV files. Playback volume was kept constant during the presentation and between raters to preserve the relative differences in voice volume between stimuli. Sets of 21 recordings (states before vacc. × after vacc. to correspond to the body odor samples and facial photographs presented during a given day) were rated by the same group of 88 raters (43 raters on Day 1, 45 on Day 2). Raters were asked to rate the attractiveness ("How attractive does the man on the voice recording sound to you?") of each target on a 7-point verbally anchored scale (from 1 – very unattractive to 7 – very attractive). Individual stimuli within the set were randomized. We have also collected data for voice healthiness but due to a technical error, these data were lost and could not be presented in this study. For schedule of the rating procedure, see Fig. 2.

Figure2

2.5 Data analyses

All statistical tests were performed using Jamovi v. 2.3.13 software. For consistency of raters' assessments, see the results of intraclass correlation (ICC) analysis in Schwambergová et al. (2021) for body odors ratings and Pátková et al. (2022) for facial images ratings.

To explore relationships in body odor characteristics (attractiveness, healthiness, intensity), facial characteristics (attractiveness and associations between colors), and voice attractiveness, we employed Spearman's correlation. Where correlation coefficients between variables were $\rho \geq 0.8$, only one of the variables was selected for subsequent analyses (Brown, 2015).

To assess changes in the perceived body odor and facial and vocal characteristics depending on the target's vaccination status (pre- vs. post-vaccination), we employed linear mixed-effects models using the GAMLj jamovi module. In all models, the rated characteristic (e.g., attractiveness or healthiness) was entered as a dependent variable and vaccination condition as the fixed-effect factor. To control for variability in donors' and raters' characteristics, we set donor and rater IDs as a random-effects factors. We used the variance of random components to estimate the contribution of each random effect to variance of the dependent variable. This results in models such as Model attractiveness <- lmer (Attractiveness ~ 1 + State (Condition) + (1|ID_rater) + (1|ID_donor)). Proportions of explained variability (pseudo R^2) for linear mixed-effect models are reported as R^2 marginal (R^2_M, proportion of variance explained by the fixed effects alone) and R^2 conditional (R^2_C, proportion of variance explained by both the fixed and random effects). Unstandardized estimates of fixed-effect slopes from linear mixed-effect models are stated with 95% confidence intervals [LL, UL]. Analogous models were used to assess the relationship between facial attractiveness and healthiness and forehead and cheek lightness, redness, and yellowness, and associations between vocal attractiveness and vocal parameters.

To test the association between CRP levels and perceived body odor and facial and vocal characteristics, we employed linear regressions. Rated characteristics were entered in pre- or post-vaccination conditions as dependent variables and with pre- or post-vaccination CRP levels as covariates.

3 RESULTS

See Table 1 for descriptive statistics of the analyzed variables, such as donors' age, height, and weight, ratings of body odor quality, facial and vocal characteristics, and CRP levels.

	Pre-vaccination			Post-vaccination		
	Mean	**SD**	**Range (min, max)**	**Mean**	**SD**	**Range (min, max)**
Age (ys)	26.19	4.62	20, 35	-	-	-
Height (cm)	181	6.74	169, 198	-	-	-
Weight (kg)	78.9	14.8	58.5, 130	-	-	-
Body odor attractiveness	3.31	1.67	1.59, 4.86*	3.62	1.7	2.24, 4.96*
Body odor intensity	4.42	1.84	3.06, 6.62*	4.25	1.9	2.63, 6.09*
Body odor healthiness	3.97	1.66	2.66, 5.12*	4.21	1.63	3.13, 5.24*
Facial attractiveness	3.08	0.978	1.37, 4.63*	2.91	0.981	1.33, 4.63*
Facial healthiness	4.38	0.932	2.23, 5.91*	4.22	0.87	2.58, 5.71*
Vocal attractiveness	3.85	1.76	1.44, 5.72*	3.84	1.71	1.88, 5.60*
CRP (mg/L)	1.32	1.31	0.2, 5.3	2.39	4.65	0.2, 21.8
Left cheek lightness L*	67.9	2.83	63.7, 74.2	68.6	2.67	64.4, 74.1
Forehead lightness L*	74.1	2.9	66.4, 80.1	74.7	2.55	65.9, 79.4
Left cheek redness a*	12.7	1.75	9.41, 15.8	12.7	1.78	9.01, 15.9
Forehead redness a*	10.2	1.77	6.54, 14.7	10	1.28	7.38, 12.6
Left cheek yellowness b*	18.2	2.54	14.2, 23.7	18.3	2.45	14.8, 23.6
Forehead yellowness b*	16.2	2.47	12.5, 20.8	16	2.42	12, 22.4
CPP (dB)	23.5	1.58	20.8, 27.4	23.1	1.54	20.5, 27.1
HNR (dB)	8.65	1.38	6.22, 10.9	8.83	1.60	6.18, 11,8
F0 (Hz)	144	33.7	103, 226	132	17.6	105, 171
F1 (Hz)	668	96.9	386, 833	673	60.7	568, 824
F2 (Hz)	1622	92.6	1494, 1838	1598	82.4	1485, 1837
F3 (Hz)	2628	150	2413, 3013	2592	124	2389, 2870
F4 (Hz)	3688	173	3445, 4084	3664	146	3468, 3983

Table 1: *Descriptive statistics for target's age, height, and weight, rating of body odor quality, facial attractiveness and healthiness, vocal attractiveness rating, color analysis, vocal analysis, and CRP before vaccination and 14 days after vaccination (N=21). Values denoted by * show mean minimum and mean maximum rating of samples.*

3.1 Relationships between variables

3.1.1 Relationship between body odor characteristics

Ratings of pre- and post-vaccination body odor characteristics were positively correlated. Odor intensity showed the strongest association ($\rho = 0.721$, $p < 0.001$, 95% CI [0.372, 0.890]), followed by healthiness ($\rho = 0.437$, $p = 0.048$, 95% CI [-0.015, 0.740]) and a comparable but not statistically significant correlation for attractiveness ($\rho = 0.418$, $p = 0.06$, 95% CI [-0.036, 0.730]). Ratings of body odor attractiveness and healthiness were positively and statistically significantly correlated both before ($\rho = 0.883$; $p < 0.001$, 95% CI [0.688, 0.960]) and after vaccination ($\rho = 0.921$; $p < 0.001$, 95% CI [0.780, 0.970]). Odor intensity rating negatively and statistically significantly correlated with both attractiveness (pre-vaccination: $\rho = -0.827$, $p < 0.001$, 95% CI [-0.937, -0.570]; post-vaccination: $\rho = -0.520$, $p = 0.02$, 95% CI [-0.789, -0.080]) and healthiness (pre-vaccination: $\rho = -0.686$, $p < 0.001$, 95% CI [-0.875, -0.320]; post-vaccination: $\rho = -0.538$, $p = 0.016$, 95% CI [-0.799, -0.110]). Because the correlation between attractiveness and healthiness reached the predefined threshold of $\rho \geq 0.8$, in subsequent analyses we used only attractiveness as a variable.

3.1.2 Relationship between facial and vocal characteristics

Ratings of pre- and post-vaccination facial characteristics were positively and statistically significantly correlated for both attractiveness ($\rho = 0.930$, $p < 0.001$, 95% CI [0.802, 0.980]) and healthiness ($\rho = 0.554$, $p = 0.009$, 95% CI [0.127, 0.810]). Ratings of perceived facial attractiveness and healthiness were also positively and statistically significantly correlated with both the pre-vaccination ($\rho = 0.706$, $p < 0.001$, 95% CI [0.348, 0.880]) and post-vaccination condition ($\rho = 0.650$, $p = 0.001$, 95% CI [0.261, 0.860]). The value of ρ did not reach the level of 0.8; in subsequent analyses we have therefore analyzed the two variables separately.

Ratings of pre- and post-vaccination vocal attractiveness were strongly positively correlated ($\rho = 0.842$, $p < 0.001$, 95% CI [0.598, 0.940]).

3.2 Relationships between all modalities

We observed no statistically significant correlation between facial and vocal attractiveness in the pre-vaccination condition ($\rho = 0.317$, $p = 0.162$, 95% CI [-0.144, 0.670]) but did find it in the post-vaccination condition ($\rho = 0.505$, $p = 0.02$, 95% CI [0.065, 0.780]). The attractiveness of body odor did not correlate statistically significantly with pre-vaccination facial ($\rho = -0.066$, $p = 0.775$, 95% CI [-0.484, 0.380]) or vocal attractiveness ($\rho = 0.342$, $p = 0.129$, 95% CI [-0.118, 0.680]), nor did it correlate with post-vaccination facial ($\rho = -0.118$, $p = 0.609$, 95% CI [-0.524, 0.330]) or vocal attractiveness ($\rho = -0.318$, $p = 0.540$, 95% CI [-0.666, 0.140]).

3.3 Changes in perception of body odor and facial and vocal attractiveness

A linear mixed-effects model showed that perceived body odor attractiveness (R^2_C = 0.261, R^2_M = 0.009) and intensity (R^2_C = 0.385, R^2_M = 0.003) were statistically significantly affected by the donor's condition (pre- vs. post-vaccination); for details, see Table 2. In particular, the ratings of body odor attractiveness were higher (by 0.31 point on the scale) and body odor intensity ratings were lower (by 0.24 point on the scale) after vaccination than before it (see Fig. 3).

We found a statistically significant effect of the target's condition (pre- vs. post-vaccination) on perceived facial attractiveness (R^2_C = 0.517, R^2_M = 0.003) and perceived healthiness (R^2_C = 0.411 R^2_M = 0.003): donors were rated as less attractive (by 0.17 point on the scale) and less healthy (by 0.16 point on the scale) after vaccination than before it (see Table 2 for details and Fig. 3).

In the case of vocal attractiveness, the model (R^2_C= 0.452, R^2_M < 0.001) showed no statistically significant effect of the donor's condition (pre- vs. post-vaccination).

Rated characteristics	**F**	**β**	**95% CI (LL, UL)**	**df**	**t**	**SE**	**p**
Body odor attractiveness	20.2	0.319	0.180, 0.458	1605.9	4.49	0.071	<0.001
Body odor intensity	8.50	-0.210	-0.351, -0.069	1606.8	-2.92	0.072	0.004
Facial attractiveness	10.4	-0.168	-0.270, - 0.066	1740	-3.23	0.052	0.001
Facial healthiness	7.07	-0.184	-0.319, - 0.048	1307.2	-2.66	0.069	0.008
Vocal attractiveness	0.01	0.002	-0.117, 0.121	1741.5	0.032	0.06	0.975

Table 2: Differences in body odor quality, facial characteristics, and vocal attractiveness in relation to the target's condition. Attractiveness ratings for odor donor ID: random components variance = 0.469, SD = 0.685, ICC = 0.181, and for odor rater ID: random components variance = 0.259, SD = 0.509 and ICC = 0.109. Odor intensity ratings for odor donor ID: random components variance = 1.092, SD = 1.045, ICC = 0.333, and for odor rater ID: random components variance = 0.268, SD = 0.518 and ICC = 0.109; healthiness ratings for odor donor ID: random components variance = 0.281, SD = 0.531, ICC = 0.117, and for odor rater ID: random components variance = 0.312, SD = 0.558 and ICC = 0.128. Attractiveness ratings for target ID: random components variance = 0.906, SD = 0.952, ICC = 0.420; for rater ID: random components variance = 0.428, SD = 0.654, ICC = 0.255. Healthiness ratings for target ID: random components variance = 0.683, SD = 0.826, ICC = 0.297; for rater ID: random components variance = 0.439, SD = 0.662, ICC = 0.213. The difference in ratings of vocal attractiveness in relation to target's condition. Attractiveness ratings for target ID: random components variance = 1.218, SD = 1.104, ICC = 0.419; for rater ID: random components variance = 0.174, SD = 0.417, ICC = 0.093.

Figure3

3.4 Changes in facial coloration

Left and right cheek measures of skin lightness (ρ = 0.801, p < 0.001), redness (ρ = 0.861, < 0.001), and yellowness (ρ = 0.925, p < 0.001) were statistically significantly positively associated. In all further analyses, we have therefore used only the left cheek color values (for details, see Supplementary Material Table S9-S16).

Linear mixed-effect models showed that lightness was not statistically significantly affected by the target's condition (pre- vs. post-vaccination) on neither the cheek (R^2_C = 0.848, R^2_M = 0.007) or the forehead (R^2_C = 0.354, R^2_M = 0.012) and neither cheek (R^2_C = 0.843, R^2_M = 3.54e-4) nor forehead (R^2_C = 0.545, R^2_M = 1.06e-4) redness were statistically significant affected by the target's condition. Target's condition also did not statistically significantly predict cheek (R^2_C = 0.850, R^2_M = 0.002) or forehead (R^2_C = 0.488, R^2_M = 9.67e-5) yellowness (for details, see Table 4).

Skin coloration	F	β	95% CI (LL, UL)	df	t	SE	p
Cheek lightness L*	1.77	0.431	-0.204, 1.07	20	1.33	0.324	0.198
Forehead lightness L*	0.736	0.58	-0.745, 1.90	20	0.858	0.676	0.401
Cheek redness a*	0.093	0.067	-0.365, 0.499	20	0.304	0.221	0.764
Forehead redness a*	0.01	0.028	-0.529, 0.585	20	0.098	0.284	0.923
Cheek yellowness b*	0.587	0.218	-0.339, 0.774	20	0.766	0.284	0.452
Forehead yellowness b*	0.008	-0.045	-1.06, 0.967	20	-0.088	0.516	0.931

Table 4: Differences in facial skin coloration depending on the target's condition. Target ID cheek lightness: random components variance = 6.11, SD = 2.47 and ICC = 0.847; forehead lightness: random components variance = 2.54, SD = 1.59 and ICC = 0.346. Target ID cheek redness: random components variance = 2.740, SD = 1.655 and ICC = 0.843; forehead redness: random components variance = 1.018, SD = 1.009 and ICC = 0.545. Target ID cheek yellowness: random components variance = 4.797, SD = 2.190 and ICC = 0.850; forehead yellowness: random components variance = 2.67, SD = 1.63 and ICC = 0.488.

3.5 Association between facial attractiveness and healthiness and facial coloration

Linear mixed-effect model testing the effect of skin color on perceived facial attractiveness before vaccination (R^2_C = 0.540, R^2_M = 0.190) showed that forehead redness was the only statistically significant predictor, and it had a negative slope. The same applies to the linear mixed-effect model of the association between skin color and perceived facial healthiness before vaccination (R^2_C = 0.491,

R^2_M = 0.182), where forehead redness negatively affected facial healthiness ratings (for more details, see Pátková et al., 2022 or Supplementary material Table S18–S21).

A linear mixed-effect model of the effect of skin color on perceived facial attractiveness after vaccination (R^2_C = 0.562 R^2_M = 0.076) showed no effect of skin color. A separate mixed-effect model had likewise shown that perceived facial healthiness after vaccination was not affected by the skin color (R^2_C = 0.461, R^2_M = 0.053). For details, see Table 5.

Characteristic	Predictors	F	β	95% CI (LL, UL)	df	t	SE	p
Facial attractiveness	Cheek lightness	0.294	0.079	- 0.206, 0.364	14.2	0.543	0.146	0.596
	Forehead lightness	0.099	0.077	- 0.404, 0.558	14.0	0.314	0.246	0.758
	Cheek redness	0.070	0.054	- 0.343, 0.450	14.6	0.264	0.2102	0.795
	Forehead redness	0.005	-0.025	- 0.742, 0.693	14.0	-0.068	0.366	0.947
	Cheek yellowness	0.292	0.088	- 0.231, 0.406	14.1	0.540	0.162	0.597
	Forehead yellowness	0.550	0.158	- 0.260, 0.576	14.0	0.742	0.213	0.471
Facial healthiness	Cheek lightness	0.025	0.021	-0.234, 0.275	13.9	0.158	0.130	0.877
	Forehead lightness	0.094	-0.068	-0.500, 0.365	14.0	-0.306	0.221	0.764
	Cheek redness	0.433	-0.118	-0.470, 0.234	14.0	-0.658	0.180	0.521
	Forehead redness	0.272	-0.171	-0.815, 0.473	13.9	-0.521	0.328	0.611
	Cheek yellowness	0.010	-0.015	-0.301, 0.272	14.1	-0.101	0.146	0.921
	Forehead yellowness	0.426	0.125	-0.250, 0.500	14.0	0.653	0.191	0.524

Table 5: *The relationship between perceived characteristics and facial colouration. Facial attractiveness ratings: for target ID, random components variance = 1.027, SD = 1.013, ICC = 0.451; for rater ID, random components variance = 0.363, SD = 0.602, ICC = 0.225. Facial healthiness ratings: for target ID, random components variance = 0.800, SD = 0.894, ICC = 0.333; for rater ID, random components variance = 0.411, SD = 0.641, ICC = 0.204.*

3.6 Changes in acoustic measures

A linear mixed-effect model showed that CPPs (R^2_C = 0.389, R^2_M = 0.016), HNR (R^2_C = 0.673, R^2_M = 0.004), F0 (R^2_C = 0.245, R^2_M = 0.048), F1 (R^2_C = 0.150, R^2_M = 0.001), F2 (R^2_C = 0.364, R^2_M = 0.018), F3 (R^2_C = 0.325, R^2_M = 0.016), and F4 (R^2_C = 0.475, R^2_M = 0.005) were not significantly affected by the target's condition (pre- vs. post-vaccination); for details see Table 6.

Characteristic	F	β	95% CI (LL, UL)	df	t	SE	p
CPP	1.07	-0.392	-1.14, 0.352	20	-1.03	0.379	0.314
HNR	0.5	0.187	-0.331, 0.706	20	0.707	0.265	0.488
F0	2.62	-12.0	-26.5, 2.51	20	-1.62	7.39	0.121
F1	0.05	5.54	-39.6, 50.6	20	0.241	23.0	0.812
F2	1.17	-23.5	-66.2, 19.1	20	-1.08	21.8	0.293
F3	1.01	-35.4	-105, 33.8	20	-1.0	35.3	0.327
F4	0.42	-23.4	-93.8, 47.0	20	-0.652	35.9	0.522

Table 6: Differences in acoustic measures depending on the target's condition. Target ID: CPPs random components variance = 0.921, SD = 0.960 and ICC = 0.379, HNR random components variance = 1.503, SD = 1.226 and ICC = 0.671, F0 random components variance = 149.0, SD = 12.2 and ICC = 0.206, F1 random components variance = 978, SD = 31.3 and ICC = 0.150, F2 random components variance = 2705, SD = 52.0 and ICC = 0.352, F3 random components variance = 5984, SD = 77.4 and ICC = 0.314, F4 random components variance = 12116, SD = 110 and ICC = 0.472 .

3.7 Association between vocal attractiveness and acoustic measures

A linear mixed-effect model showed that pre-vaccination vocal attractiveness was statistically significantly affected by the target's CPPs ($R^2_C = 0.453$, $R^2_M = 0.06$). Higher CPPs predicted a target's voice being rated as more attractive (for details, see Table 7).

Characteristic	**Parameters**	**F**	**β**	**95% CI (LL, UL)**	**df**	**t**	**SE**	**p**
Vocal attractiveness	CPPs	9.89	0.206	0.077, 0.334	307.6	3.145	0.065	0.002
	HNR	2.96	-0.092	-0.197, 0.012	978.3	-1.72	0.053	0.085
	F0	0.89	-0.011	-0.03, 0.012	237.7	-0.94	-0.011	0.347
	F1	0.387	-0.0009	-0.004, 0.002	327.9	-0.622	0.001	0.534
	F2	0.187	0.001	-0.004, 0.006	508.7	0.432	0.002	0.666
	F3	1.557	0.001	-0.0008, 0.004	1214.9	1.248	0.001	0.212
	F4	3.526	-0.001	-0.004, 0.0008	641.0	-1.878	0.001	0.061

Table 7: Relationships between acoustic measures (CPPs, HNR, F1-F4 and F0) and perceived vocal attractiveness. For target ID: random components variance = 1.031, SD = 1.015, ICC = 0.380; for rater ID: random components variance = 0.176, SD = 0.420, ICC = 0.094.

3.8 Relationship between CRP and body odor, face, and voice ratings

Interestingly, the mean CRP levels did not statistically significantly differ between the two conditions (pre- vs. post-vaccination; $F(1, 20) = 1.41$, $\beta = 1.06$, 95% CI [-0.690, 2.81], $p = 0.249$ ($R^2_C = 0.297$, $R^2_M = 0.024$). Nevertheless, pre-vaccination CRP levels were negatively associated with pre-vaccination body odor attractiveness ($F(1, 19) = 6.43$, $\beta = -0.291$, 95% CI [-0.531, -0.05], $p = 0.02$, $R^2 = 0.213$) and positively predicted by pre-vaccination body odor intensity ($F(1, 19) = 5.48$, $\beta = 0.396$, 95% CI [0.042, 0.750], $p = 0.03$, $R^2 = 0.224$). Interestingly, though, post-vaccination CRP levels predicted neither post-vaccination body odor attractiveness ($F(1, 19) = 0.025$, $\beta = 0.006$, 95% CI [-0.08, 0.09], $p = 0.876$, $R^2 = 0.001$) nor post-vaccination odor intensity ($F(1, 19) = 0.015$, $\beta = -0.007$, 95% CI [-0.130, 0.115], $p = 0.904$, $R^2 = 0.0007$).

Moreover, pre-vaccination CRP levels negatively predicted pre-vaccination facial attractiveness rating (F (1, 19) = 8.85, β = -0.419, 95% CI [-0.714, -0.124], p = 0.008, R^2 = 0.318) but not the pre-vaccination healthiness rating (F (1,19) = 1.13, β = -0.168, 95% CI [-0.498, 0.163], p = 0.302, R^2 = 0.060). We found no relationship between post-vaccination CRP levels and post-vaccination perceived facial attractiveness (F (1,19) = 1.63, β = -0.06, 95% CI [-0.157, 0.04], p = 0.217, R^2 = 0.079) or healthiness (F (1,19) = 0.430, β = -0.027, 95% CI [-0.117, 0.061], p = 0.520, R^2 = 0.022).

We found no relationship between pre-vaccination CRP levels and pre-vaccination perceived vocal attractiveness (F (1,19) = 2.39, β = -0.301, 95% CI [-0.710, 0.107], p = 0.139, R^2 = 0.112). An analogous result was observed in the post-vaccination condition (F (1,19) = 0.739, β = -0.117, 95% CI [-0.213, -0.02], p = 0.401, R^2 = 0.039).

4 DISCUSSION

The aim of this study was to test whether immunoactivation affects perceived characteristics of body odor, face, and voice. We stimulated the immune system activation using vaccines against viral and bacterial agents (hepatitis A/B and meningococcus) and collected body odor samples, facial photographs, and voice recordings before vaccination and 14 days after it.

Contrary to our expectations, we found that body odor samples were rated as more attractive and less intense 14 days after vaccination. The opposite effect, which was in line with predictions, was observed in the ratings of facial characteristics, where facial photographs were perceived as less attractive and less healthy after vaccination, while vocal attractiveness did not differ between the pre- and post-vaccination conditions. Interestingly, pre-vaccination CRP levels were negatively associated with body odor and facial attractiveness ratings. The effect sizes in our study were rather small, ranging mostly between 0.10 and 0.29. The largest post-vaccination effect size was found for body odor attractiveness (an increase by 0.31 points on a 7-point scale), followed by the change in body odor intensity (a decrease of 0.17 points), facial attractiveness (a decrease of 0.17 points), and facial healthiness (a decrease of 0.16 points). Concerning the CRP results, the largest effect size was found for facial attractiveness (β = -0.419), meaning that for each one-unit increase in the CRP, facial attractiveness decreased by 0.419 units. The results were similar for body odor attractiveness (β = -0.291), meaning that for each one- unit increase in the CRP, facial attractiveness decreased by 0.291 units. Moreover, CRP variation explains between 21–31.8% of variance of perceived attractiveness. .

Olfactory cues may be helpful because they can often be assessed from a distance, thus allowing others to avoid potential infection. Moreover, these cues can be perceived from the environment and under conditions where other senses (hearing and sight) are impaired. The results of previous animal and human studies show a decrease in preference for the odor of sick individuals (e.g., Kavaliers & Colwell, 1995; Kavaliers et al., 2003a; Arakawa et al., 2009; Moshkin et al., 2012; Olsson et al., 2014; Sarolidou et al., 2020), often shortly after the onset of immune reaction to illness or even in cases where symptoms of a disease are no longer present. For example, men who recovered after the acute stage of gonorrhea were more likely to be associated with a floral smell (Moshkin et al., 2012). The impact of infection on body odor can be tested by comparing odor samples from a person collected when the person is ill and the same person is healthy, but this approach is logistically challenging and the variability of naturally occurring diseases is high. This is why in experimental conditions, researchers try to simulate a disease by administering an LPS injection which activates the innate immune response (e.g., Henderson et al., 2017; Olsson et al., 2014). An alternative method is vaccination (Shattuck & Muehlenbein, 2015) whose application can induce side effects comparable to the symptoms of a disease (Di Pasquale et al., 2016), because in both conditions the immune response is activated. Although negative changes in body odor could occur within a few hours (Olsson et al., 2014) or days (Sarolidou et al., 2020) after immunoactivation by vaccination, our aim was to wait for a sufficient increase in specific antibodies (see Schwambergová et al., 2021). Therefore, we collected our samples two weeks after vaccination, at a time when one could expect the immune reaction to peak (Palm & Medzhitov, 2007). One may speculate that when the putative negative effects of immune system activation diminish and body odor returns to its baseline quality, this positive change may be at some point magnified, perhaps just about two weeks after immunization. A positive shift in the attractiveness of body odor after activation of the immune system could thus serve as an indicator of a higher-quality male who can successfully cope with a disease.

When it comes to judgments of attractiveness, visual perception is in humans usually considered the most crucial. In several previous studies, facial photographs of participants after activation of the immune system were rated as less attractive (Axelsson et al., 2018; Regenbogen et al., 2017). Moreover, these studies have shown that raters can discriminate between individuals with an activated immune system and healthy controls within a few hours after vaccination based on their faces (Arshamian et al., 2021). Among visual facial characteristics, skin coloration is the most sensitive to changes associated with illness (Henderson et al., 2017; Cai et al., 2019). Our results show that faces were rated as less attractive and less healthy two weeks after vaccination, although we found no statistically significant changes in post-vaccination skin color as measured directly *in vivo* using a spectrophotometer or from photographs. The explanation thus cannot rely solely on changes in the lightness, redness, or yellowness of the skin. One might argue that raters could notice other cues not analyzed in this study, such as skin texture, drooping mouth corners or degraded hair quality after vaccination, which in turn may affect attractiveness and healthiness judgments.

The acoustic modality in our study showed no statistically significant change in attractiveness ratings between the pre- and post-vaccination condition. We also found no differences in voice acoustics, such as a smoothed cepstral peak prominence (CPP), which was previously shown to be the best acoustic predictor of perceptual voice quality (Eadie & Baylor, 2006), or the fundamental frequency (F0). Previous studies suggest that infections and immune activity may affect vocal acoustics. For example, male mice shortly after LPS administration produced a lower number of regular ultrasonic syllables and a larger number of high-frequency ultrasonic syllables (Lopes & König, 2016). In humans, fundamental frequency negatively influenced healthiness ratings, although raters were not able to accurately assess the health status of male speakers based on their voices (Albert et al., 2021). It is likely that changes in the voice and other sounds are linked to symptoms of acute illness, such as hoarseness, sneezing, or coughing, which are rated as disgusting noises (Michalak et al., 2020). During the voice recordings, our participants did not exhibit any such symptoms in the post-vaccination condition. Like in previous studies, we found that CPPs affected attractiveness ratings positively (Balasubramanium et al., 2012).

Attractive characteristics are frequently considered to serve as cues to individual's health status perceptible by our senses. But the patterns of our findings regarding changes in ratings before and after vaccination differed between the three selected modalities. Multisensory perception plays an important role in overall perception of others because deployment of multiple sensory channels can yield a more reliable assessment. In general, multimodal perception has been considered in the context of two main hypothesis: the 'multiple messages' hypothesis, which proposes that each cue or signal provides unique and independent (i.e. non-redundant) information about the individual's condition and quality, or the 'backup signals' hypothesis, according to which cues or signals provide similar and overlapping (i.e. redundant) information (Möller and Pomiankowski, 1993). There is strong evidence that a combination of faces with voices or odors – as opposed to presenting each of these modalities separately – can significantly affect judgements of overall attractiveness (Ferdenzi et al., 2016; Regenbogen et al., 2017). Although the visual modality is considered the most important in humans, other modalities may serve as additional sources for the formation of the overall judgment (Groyecka et al., 2017). Our results can be interpreted as rather in line with the multiple messages hypothesis, because every modality may have carried different information about the individual's condition two weeks after vaccination, and this was reflected in the ratings (Třebický et al., 2023).

Furthermore, our results demonstrated a high sensitivity of human smell and sight to subtle cues of inflammation. CRP is a marker of inflammation processes and its elevation reliably shows currently ongoing infection and/or inflammation in the body. In the pre-vaccination condition, CRP levels of our sample did not exceed 5.5 mg/L, that is, levels considered clinically insignificant and normal. Such variations ~ 5.5 mg/L may be caused by a small local inflammation. On the other hand, there are also other factors that can affect CRP variation, such as age, sex, smoking status, weight, lipid levels, and blood pressure (Sproston & Ashworth, 2018). Still, our results showed a negative relationship between pre-vaccination CRP levels and perceived body odor and facial attractiveness, which suggests that even a subtle increase in CRP levels can be perceived by smell or sight. This negative association was found only in the pre-vaccination condition, which may indicate that vaccination may temporarily disrupt the naturally occurring links between normal CRP levels and the perception of current health status.

Although most studies focus on the perception of health in the context of mate choice, identification of infected conspecifics is beneficial not only during selection of potential mates. Although most studies investigate the perception of healthiness in the context of mate choice, identification of infected conspecifics is beneficial not only during selection of potential mates. In the context of mate choice, it is the between-individual differences in health cues that might be of particular relevance. In contrast, in interactions with non-strangers, the within-individual variation in current health might be more relevant because it may help to avoid acutely ill individuals and thus lower the risk of infection. Detection of various cues to threats in the environment and within the social group is the cornerstone of complex avoidance mechanisms and it can lead to behaviors that lessen the risk of contagion. This 'behavioral immune system' consists of psychological mechanisms responsible for avoidance behavior (Schaller & Park, 2011). The main task of this system is to detect possible contaminants, elicit affective reactions, and facilitate avoidance of prolonged exposure to pathogen sources. Our results provide some support for the function of behavioral immune system in terms of modulation of perceived attractiveness after immunoactivation.

4.1 Limitations

Although comparable with previous studies on the perception of body odor quality (see Moshkin et al., 2012 or Regenbogen et al., 2017), an unfortunate limitation of the present study is the low sample size of sample donors. We faced considerable difficulties in recruiting participants mostly because of their hesitation to participate in a 'vaccination' study due to the anti-vaccination movement (even before Covid-19 pandemic). Among those willing to volunteer, it was also difficult to find those who met all the inclusion criteria, chiefly that of not being vaccinated against hepatitis A/B or meningococcus in the past ten years. Vaccination against hepatitis B has been included in the compulsory vaccination protocol in the Czech Republic in 2001 (e.g., Bozzola et al., 2018) and revaccination in adolescence is also highly recommended (Shepard et al., 2006). Furthermore, there was no control group of donors (injected with an empty solvent or saline) who could be compared to the (vaccinated) experimental group in terms of changes in odor, facial, and vocal cues. This is because our sample size was already limited and splitting it into two groups would considerably lower the statistical power of the study. Future studies should certainly include a control group whose results would help interpret the temporal fluctuations in the various followed characteristics.

Furthermore, the project focused mainly on humoral adaptive immunity, in particular the increase of specific antibodies after vaccination, and its effect on body odor quality (see Schwambergová et al., 2021) and perceived facial attractiveness (see Pátková et al, 2022). It should be noted that the most distinctive changes in body odor, face, or voice may take place shortly after administration of a vaccine (typically a few hours to a few days after injection), as demonstrated by studies that used LPS (Olsson et al., 2014; Henderson et al., 2017). The choice of sampling two weeks after vaccination may thus be appropriate for assessing the increase in antibody levels – which was in fact our main goal in two other studies based on the same dataset (Schwambergová et al., 2021 and Pátková et al., 2022) – but not for assessing the perceptual cues linked to acute sickness.

4.2 Conclusions

The aim of this study was to test the role of multiple sensory cues in assessing the current health status of individuals. Our findings show that changes in the perceived qualities of body odor and facial attractiveness after vaccination do take place. Body odor attractiveness increased and facial attractiveness decreased 14 days after vaccination compared to the pre-vaccination state. These results can be interpreted as providing support for the multiple messages hypothesis, because every modality may have carried different information regarding the individual’s condition. Moreover, we found that pre-vaccination CRP levels negatively predicted body odor and facial attractiveness, which shows that even subtle changes within relatively low CRP levels can manifest as slight changes in body odor and facial appearance. This ability to distinguish minor nuances in the health status may help in distinguishing healthier mates and social partners.

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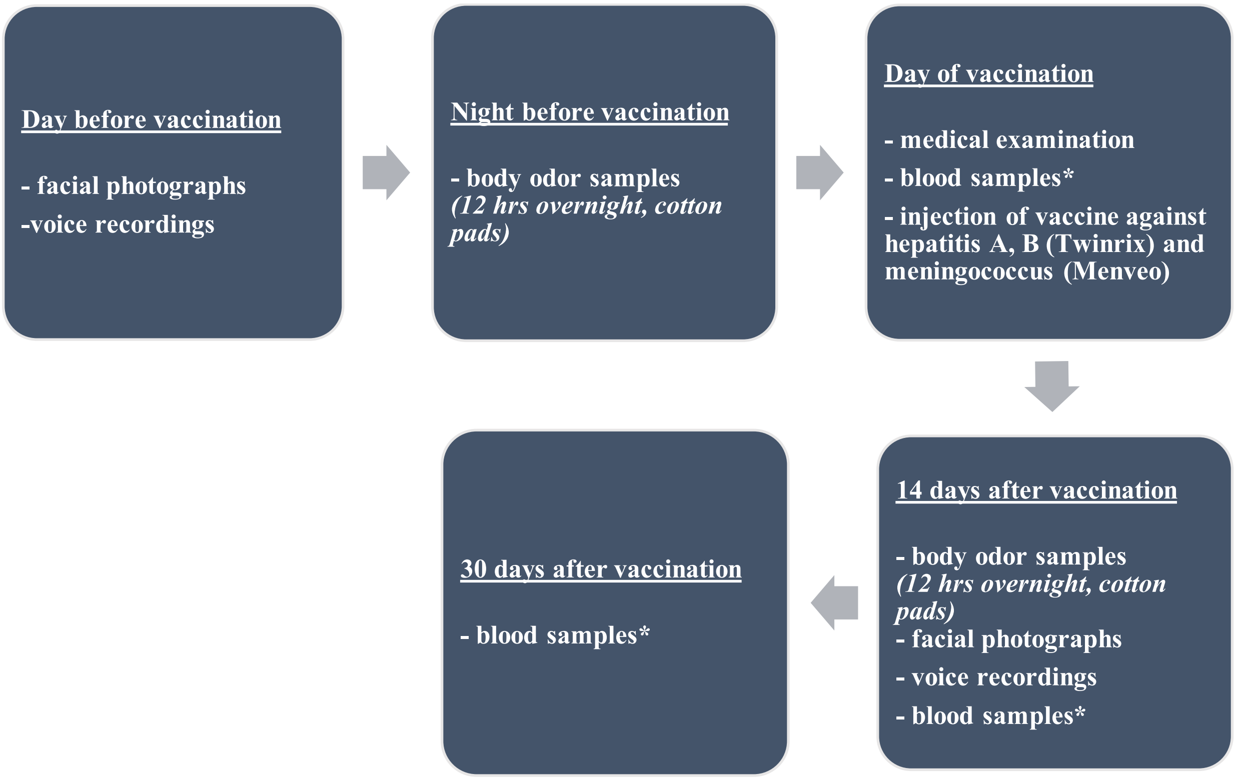

Fig. 1: Study schedule step by step. Approx. 48 hours before body odor collection, targets refrained from spicy and aromatic foods, alcohol, and increased physical activity. *Blood samples were collected to assess levels of specific antibodies, steroid hormones, and CRP.

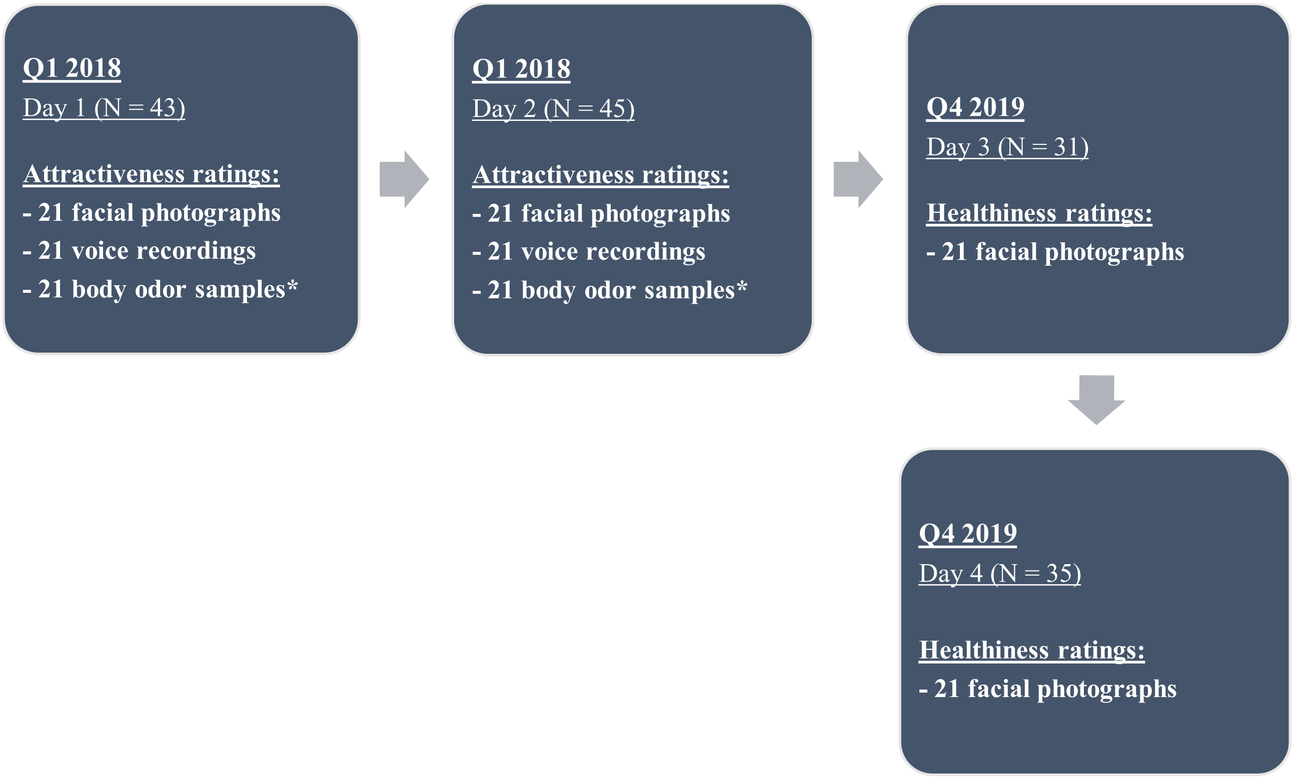

Fig. 2: Schedule of the rating procedure. All stimuli were rated on a 7-point scale for attractiveness (*body odor samples were rated for attractiveness, intensity, and healthiness at once) and healthiness. Stimuli from targets were presented to a rater on a given day either in the pre- or the post-vaccination condition.

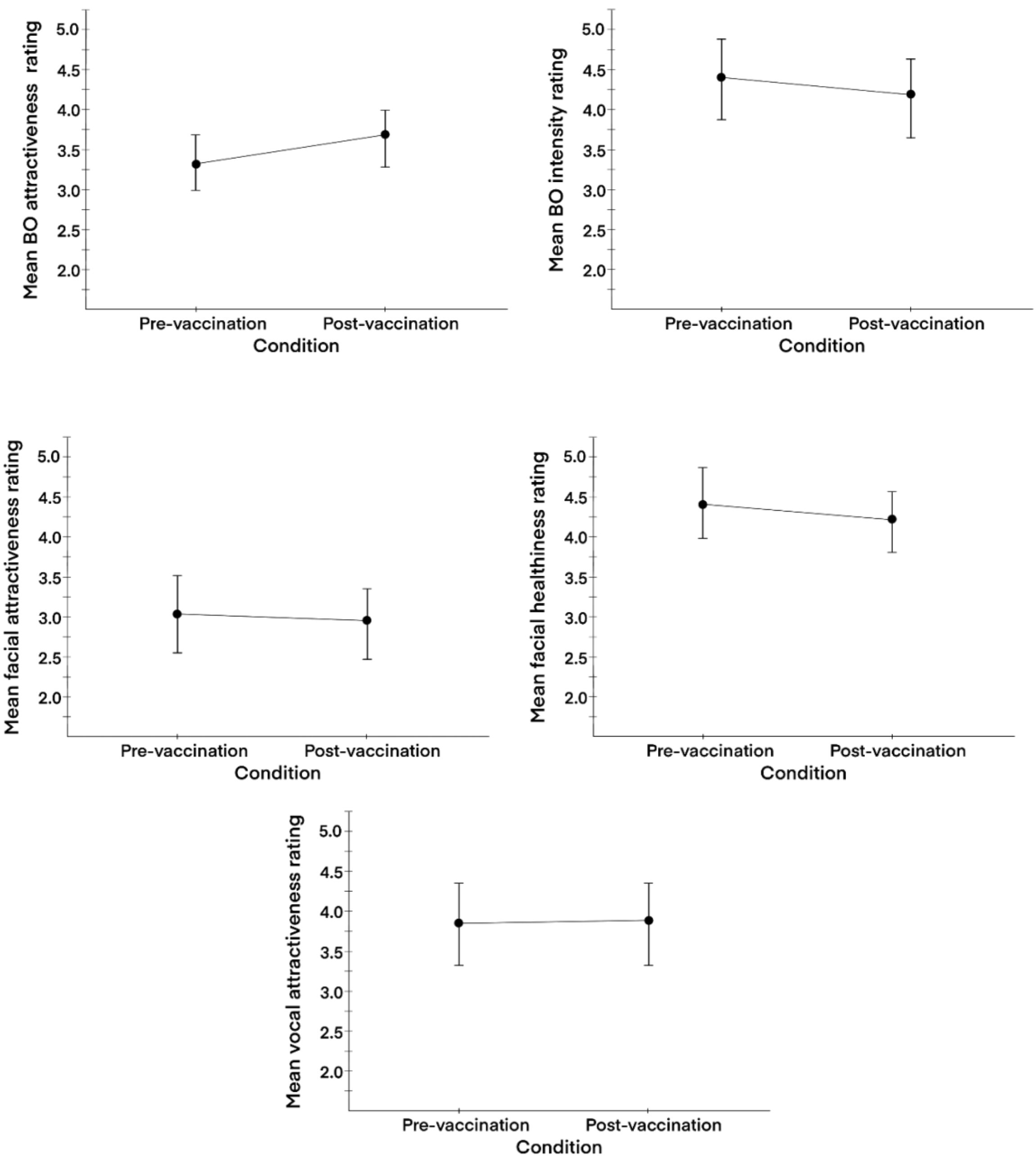

Fig. 3: Attractiveness (A) and intensity (B) of body odor samples, attractiveness (C) and healthiness (D) of faces and attractiveness of voices (E) depending on the condition (pre- vs. post-vaccination). Error bars show 95% confidence intervals.

Table S1 Correlation between individual color measurements of right and left cheek and forehead from spectrophotometer before vaccination

		Right cheek lightness	Left cheek lightness	Forehead lightness	Right cheek redness	Left cheek redness	Forehead redness	Right cheek yellowness	Left cheek yellowness	Forehead yellowness
Right cheek lightness	Spearman's rho	—								
	p-value	—								
Left cheek lightness	Spearman's rho	0.492 *	—							
	p-value	0.029	—							
Forehead lightness	Spearman's rho	0.236	0.262	—						
	p-value	0.315	0.264	—						
Right cheek redness	Spearman's rho	-0.574 **	-0.104	0.081	—					
	p-value	0.009	0.663	0.733	—					
Left cheek redness	Spearman's rho	-0.298	-0.564 *	0.09	0.414	—				
	p-value	0.202	0.011	0.705	0.071	—				
Forehead redness	Spearman's rho	-0.121	-0.226	-0.599 **	0.068	0.32	—			
	p-value	0.611	0.337	0.005	0.774	0.168	—			
Right cheek yellowness	Spearman's rho	-0.012	-0.002	-0.328	-0.131	-0.002	0.134	—		
	p-value	0.962	0.997	0.158	0.581	0.997	0.574	—		
Left cheek yellowness	Spearman's rho	-0.314	0.162	-0.329	0.132	-0.119	0.105	0.741 ***	—	
	p-value	0.177	0.492	0.156	0.577	0.617	0.661	< .001	—	
Forehead yellowness	Spearman's rho	-0.117	0.218	0.262	0.283	0.137	0.15	0.462 *	0.487 *	—
	p-value	0.621	0.354	0.264	0.226	0.564	0.527	0.042	0.031	—

Note. * p < .05. ** p < .01. *** p < .001

Table S2 Correlation between individual color measurements of right and left cheek and forehead from spectrophotometer after vaccination

		Right cheek lightness	Left cheek lightness	Forehead lightness	Right cheek redness	Left cheek redness	Forehead redness	Right cheek yellowness	Left cheek yellowness	Forehead yellowness
Right cheek lightness	Spearman's rho	—								
	p-value	—								
Left cheek lightness	Spearman's rho	0.662 **	—							
	p-value	0.002	—							
Forehead lightness	Spearman's rho	0.386	0.323	—						
	p-value	0.093	0.164	—						
Right cheek redness	Spearman's rho	-0.638 **	-0.475 *	-0.129	—					
	p-value	0.003	0.036	0.586	—					
Left cheek redness	Spearman's rho	-0.426	-0.621 **	0.06	0.707 ***	—				
	p-value	0.063	0.004	0.802	< .001	—				
Forehead redness	Spearman's rho	-0.368	-0.245	-0.368	0.514 *	0.352	—			
	p-value	0.111	0.296	0.111	0.022	0.129	—			
Right cheek yellowness	Spearman's rho	0.017	0.026	-0.08	0.334	0.323	0.062	—		
	p-value	0.947	0.916	0.738	0.15	0.164	0.797	—		
Left cheek yellowness	Spearman's rho	-0.167	0.102	-0.191	0.226	-0.05	-0.023	0.567 *	—	
	p-value	0.48	0.667	0.418	0.337	0.836	0.927	0.01	—	
Forehead yellowness	Spearman's rho	-0.227	-0.026	0.224	0.119	0.021	-0.379	0.22	0.6 **	—
	p-value	0.334	0.916	0.341	0.617	0.932	0.1	0.351	0.006	—

Note. * p < .05. ** p < .01. *** p < .001

Table S3 Differences between separate measurements from the right and left cheek and forehead for lightness before vaccination (spectrophotometer)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	2.12	2	37.2	0.134

Group Descriptives

	Place	N	Mean	SD	SE
value	Right cheek	20	62.5	2.35	0.525
	Left cheek	20	62.1	1.9	0.424
	Forehead	20	60.9	2.65	0.593

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead
Right cheek	Mean difference	—	0.401	1.6
	t-value	—	0.546	2.17
	df	—	57	57
	p-value	—	0.849	0.084
Left cheek	Mean difference		—	1.2
	t-value		—	1.63
	df		—	57
	p-value		—	0.242
Forehead	Mean difference			—
	t-value			—
	df			—
	p-value			—

Note. * p < .05. ** p < .01. *** p < .001

Table S4 Differences between separate measurements from the right and left cheek and forehead for lightness after vaccination (spectrophotometer)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	2.01	2	37.8	0.148

Group Descriptives

	Place	N	Mean	SD	SE
value	Right cheek	20	63.4	2.11	0.471
	Left cheek	20	62.8	1.85	0.413
	Forehead	20	62	2.15	0.482

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead
Right cheek	Mean difference	—	0.528	1.353
	t-value	—	0.818	2.1
	df	—	57	57
	p-value	—	0.693	0.1
Left cheek	Mean difference		—	0.825
	t-value		—	1.28
	df		—	57
	p-value		—	0.413
Forehead	Mean difference			—
	t-value			—
	df			—
	p-value			—

Note. * p < .05. ** p < .01. *** p < .001

Table S5 Differences between separate measurements from the right and left cheek and forehead for redness before vaccination (spectrophotometer)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	2.53	2	37.7	0.093

Group Descriptives

	Value	N	Mean	SD	SE
value	Right cheek	20	15.9	3.4	0.761
	Left cheek	20	17.3	3.16	0.706
	Forehead	20	18.2	2.79	0.625

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead
Right cheek	Mean difference	—	-1.39	-2.233
	t-value	—	-1.4	-2.259
	df	—	57	57
	p-value	—	0.347	0.07
Left cheek	Mean difference		—	-0.847
	t-value		—	-0.857
	df		—	57
	p-value		—	0.67
Forehead	Mean difference			—
	t-value			—
	df			—
	p-value			—

Note. * p < .05. ** p < .01. *** p < .001

Table S6 Differences between separate measurements from the right and left cheek and forehead for redness after vaccination (spectrophotometer)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	1.97	2	37.3	0.153

Group Descriptives

	Value	N	Mean	SD	SE
value	Right cheek	20	16.9	3.45	0.772
	Left cheek	20	17.3	2.63	0.588
	Forehead	20	18.6	2.38	0.533

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead
Right cheek	Mean difference	—	-0.385	-1.64
	t-value	—	-0.426	-1.81
	df	—	57	57
	p-value	—	0.905	0.175
Left cheek	Mean difference		—	-1.25
	t-value		—	-1.38
	df		—	57
	p-value		—	0.356
Forehead	Mean difference			—
	t-value			—
	df			—
	p-value			—

Note. * p < .05. ** p < .01. *** p < .001

Table S7 Differences between separate measurements from the right and left cheek and forehead for yellowness before vaccination (spectrophotometer)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	0.315	2	37.8	0.731

Group Descriptives

	Value	N	Mean	SD	SE
value	Right cheek	20	9.85	2.04	0.457
	Left cheek	20	10.27	2	0.447
	Forehead	20	10.29	1.73	0.386

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead
Right cheek	Mean difference	—	-0.421	-0.4436
	t-value	—	-0.69	-0.7274
	df	—	57	57
	p-value	—	0.77	0.748
Left cheek	Mean difference		—	-0.0226
	t-value		—	-0.037
	df		—	57
	p-value		—	0.999
Forehead	Mean difference			—
	t-value			—
	df			—
	p-value			—

Note. * p < .05. ** p < .01. *** p < .001

Table S8 Differences between separate measurements from the right and left cheek and forehead for yellowness after vaccination (spectrophotometer)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	0.404	2	38	0.67

Group Descriptives

	Value	N	Mean	SD	SE
value	Right cheek	20	9.3	1.56	0.348
	Left cheek	20	9.57	1.47	0.329
	Forehead	20	9.73	1.47	0.328

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead
Right cheek	Mean difference	—	-0.274	-0.43
	t-value	—	-0.578	-0.908
	df	—	57	57
	p-value	—	0.832	0.638
Left cheek	Mean difference		—	-0.156
	t-value		—	-0.329
	df		—	57
	p-value		—	0.942
Forehead	Mean difference			—
	t-value			—
	df			—
	p-value			—

Note. * p < .05. ** p < .01. *** p < .001

Table S9 Correlation between individual color measurements of right and left cheek and forehead from photos before vaccination

		Right cheek lightness	Left cheek lightness	Forehead lightness	Right cheek redness	Left cheek redness	Forehead redness	Right cheek yellowness	Left cheek yellowness	Forehead yellowness
Right cheek lightness	Spearman's rho	—								
	p-value	—								
Left cheek lightness	Spearman's rho	0.801 ***	—							
	p-value	< .001	—							
Forehead lightness	Spearman's rho	0.481 *	0.444 *	—						
	p-value	0.027	0.044	—						
Right cheek redness	Spearman's rho	-0.611 **	-0.397	-0.324	—					
	p-value	0.003	0.075	0.152	—					
Left cheek redness	Spearman's rho	-0.53 *	-0.475 *	-0.297	0.861 ***	—				
	p-value	0.014	0.03	0.191	< .001	—				
Forehead redness	Spearman's rho	-0.453 *	-0.428	-0.468 *	0.558 **	0.544 *	—			
	p-value	0.039	0.053	0.032	0.009	0.011	—			
Right cheek yellowness	Spearman's rho	-0.324	-0.354	-0.336	-0.093	-0.155	0.318	—		
	p-value	0.152	0.115	0.137	0.689	0.501	0.16	—		
Left cheek yellowness	Spearman's rho	-0.432	-0.435 *	-0.248	0.127	0.011	0.322	0.925 ***	—	
	p-value	0.05	0.049	0.279	0.584	0.962	0.155	< .001	—	
Forehead yellowness	Spearman's rho	-0.313	-0.194	-0.562 **	0.171	-0.063	0.097	0.68 ***	0.689 ***	—
	p-value	0.168	0.399	0.008	0.459	0.786	0.676	< .001	< .001	—

Note. * p < .05. ** p < .01. *** p < .001

Table S10 Correlation between individual color measurements of right and left cheek and forehead from photos after vaccination

		Right cheek lightness		Left cheek lightness		Forehead lightness		Right cheek redness		Left cheek redness	Forehead redness	Right cheek yellowness		Left cheek yellowness		Forehead yellowness
Right cheek lightness	Spearman's rho	—														
	p-value	—														
Left cheek lightness	Spearman's rho	0.721	***	—												
	p-value	< .001		—												
Forehead lightness	Spearman's rho	0.481	*	0.464	*	—										
	p-value	0.029		0.036		—										
Right cheek redness	Spearman's rho	-0.594	**	-0.473	*	-0.297		—								
	p-value	0.005		0.032		0.190		—								
Left cheek redness	Spearman's rho	-0.482	*	-0.508	*	-0.294		0.873	***	—						
	p-value	0.028		0.020		0.196		< .001		—						
Forehead redness	Spearman's rho	-0.183		-0.305		-0.792	***	0.255		0.284	—					
	p-value	0.425		0.178		< .001		0.264		0.211	—					
Right cheek yellowness	Spearman's rho	-0.196		-0.227		-0.155		-0.164		-0.194	-0.148	—				
	p-value	0.393		0.320		0.502		0.477		0.399	0.520	—				
Left cheek yellowness	Spearman's rho	-0.004		-0.194		-0.134		-0.223		-0.236	-0.100	0.922	***	—		
	p-value	0.989		0.399		0.562		0.329		0.301	0.665	< .001		—		
Forehead yellowness	Spearman's rho	-0.217		-0.204		-0.366		0.166		0.229	-0.027	0.645	**	0.653	**	—
	p-value	0.343		0.374		0.103		0.470		0.317	0.908	0.002		0.002		—

Note. * p < .05. ** p < .01. *** p < .001

Table S11 Differences between separate measurements from the right and left cheek and forehead for lightness before vaccination (photos)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	27.6	2	39.8	< .001

Group Descriptives

	Place	N	Mean	SD	SE
value	Right cheek	21	69.2	2.39	0.525
	Left cheek	21	68.1	2.7	0.424
	Forehead	21	74.2	2.86	0.593

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead	
Right cheek	Mean difference	—	1.03	-4.98	***
	t-value	—	1.26	-6.08	
	df	—	60	60	
	p-value	—	0.424	< .001	
Left cheek	Mean difference		—	-6.01	***
	t-value		—	-7.34	
	df		—	60	
	p-value		—	< .001	
Forehead	Mean difference			—	
	t-value			—	
	df			—	
	p-value			—	

Note. * p < .05. ** p < .01. *** p < .001

Table S12 Differences between separate measurements from the right and left cheek and forehead for lightness after vaccination (photos)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	31	2	39.5	< .001

Group Descriptives

	Place	N	Mean	SD	SE
value	Right cheek	21	70.5	2.11	0.46
	Left cheek	21	68.6	2.67	0.583
	Forehead	21	74.7	2.55	0.556

Tukey Post-Hoc Test – value

		Right cheek	Left cheek		Forehead	
Right cheek	Mean difference	—	1.91	*	-4.25	***
	t-value	—	2.52		-5.61	
	df	—	60		60	
	p-value	—	0.038		< .001	
Left cheek	Mean difference		—		-6.16	***
	t-value		—		-8.13	
	df		—		60	
	p-value		—		< .001	
Forehead	Mean difference				—	
	t-value				—	
	df				—	
	p-value				—	

Note. * p < .05. ** p < .01. *** p < .001

Table S13 Differences between separate measurements from the right and left cheek and forehead for redness before vaccination (photos)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	17	2	39.4	< .001

Group Descriptives

	Value	N	Mean	SD	SE
value	Right cheek	21	12.34	1.91	0.417
	Left cheek	21	12.62	1.83	0.398
	Forehead	21	9.98	1.45	0.316

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead	
Right cheek	Mean difference	—	-0.28	2.36	***
	t-value	—	-0.521	4.39	
	df	—	60	60	
	p-value	—	0.861	< .001	
Left cheek	Mean difference		—	2.64	***
	t-value		—	4.91	
	df		—	60	
	p-value		—	< .001	
Forehead	Mean difference			—	
	t-value			—	
	df			—	
	p-value			—	

Note. * p < .05. ** p < .01. *** p < .001

Table S14 Differences between separate measurements from the right and left cheek and forehead for redness after vaccination (photos)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	18.9	2	39.1	< .001

Group Descriptives

	Value	N	Mean	SD	SE
value	Right cheek	21	12.1	1.69	0.368
	Left cheek	21	12.7	1.78	0.389
	Forehead	21	10	1.28	0.279

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead	
Right cheek	Mean difference	—	-0.607	2.07	***
	t-value	—	-1.23	4.2	
	df	—	60	60	
	p-value	—	0.439	< .001	
Left cheek	Mean difference		—	2.68	***
	t-value		—	5.43	
	df		—	60	
	p-value		—	< .001	
Forehead	Mean difference			—	
	t-value			—	
	df			—	
	p-value			—	

Note. * p < .05. ** p < .01. *** p < .001

Table S15 Differences between separate measurements from the right and left cheek and forehead for yellowness before vaccination (photos)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	4.34	2	39.8	0.02

Group Descriptives

	Value	N	Mean	SD	SE
value	Right cheek	21	17.2	1.96	0.427
	Left cheek	21	18.1	2.3	0.502
	Forehead	21	16	2.25	0.492

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead	
Right cheek	Mean difference	—	-0.89	1.19	
	t-value	—	-1.33	1.77	
	df	—	60	60	
	p-value	—	0.387	0.19	
Left cheek	Mean difference		—	2.07	**
	t-value		—	3.09	
	df		—	60	
	p-value		—	0.008	
Forehead	Mean difference			—	
	t-value			—	
	df			—	
	p-value			—	

Note. * p < .05. ** p < .01. *** p < .001

Table S16 Differences between separate measurements from the right and left cheek and forehead for yellowness after vaccination (photos)

One-Way ANOVA (Welch's)

	F	df1	df2	p
value	4.82	2	39.6	0.013

Group Descriptives

	Value	N	Mean	SD	SE
value	Right cheek	21	17.3	2	0.436
	Left cheek	21	18.3	2.45	0.535
	Forehead	21	16	2.42	0.528

Tukey Post-Hoc Test – value

		Right cheek	Left cheek	Forehead	
Right cheek	Mean difference	—	-0.978	1.36	
	t-value	—	-1.38	1.92	
	df	—	60	60	
	p-value	—	0.359	0.143	
Left cheek	Mean difference		—	2.34	**
	t-value		—	3.29	
	df		—	60	
	p-value		—	0.005	
Forehead	Mean difference			—	
	t-value			—	
	df			—	
	p-value			—	

Note. * p < .05. ** p < .01. *** p < .001

Table S17 Differences in facial coloration before and after vaccination measured by spectrophotometer

Cheek lightness: R-squared conditional = 0.488. R-squared marginal = 0.045
Forehead lightness: R-squared conditional = 0.466. R-squared marginal = 0.0522
Cheek redness: R-squared conditional = 0.796. R-squared marginal = 0.015
Forehead redness: R-squared conditional = 0.657. R-squared marginal = 0.024
Cheek yellowness: R-squared conditional = 0.472. R-squared marginal = 0.002
Forehead yellowness: R-squared conditional = 0.245. R-squared marginal = 3.82e-7

Characteristic	Parameters	F	β	95% CI (LL. UL)	df	t	SE	p
Cheek lightness	Condition	3.25	0.804	-0.067. 1.68	18	1.8	0.446	0.088
Forehead lightness	Condition	3.62	1.06	-0.032. 2.15	18	1.9	0.557	0.073
Cheek redness	Condition	2.75	-0.400	-0.872. 0.073	18	-1.66	0.241	0.115
Forehead redness	Condition	2.59	-0.490	-1.09. 0.107	18	-1.61	0.305	0.125
Cheek yellowness	Condition	0.128	-0.246	-1.60. 1.10	18	-0.358	0.689	0.725
Forehead yellowness	Condition	1.87E-05	0.003	-1.36. 1.36	18	0.004	0.693	0.997

Target ID cheek lightness random components variance = 1.63. SD = 1.28 and ICC = 0.464. forehead lightness random components variance = 2.28. SD = 1.51 and ICC = 0.437. Target ID cheek redness random components variance = 2.109. SD = 1.452 and ICC = 0.793. forehead redness random components variance = 1.627. SD = 1.276 and ICC = 0.649. Target ID left cheek yellowness random components variance = 4.02. SD = 2.00 and ICC = 0.471. forehead yellowness random components variance = 1.48. SD = 1.22 and ICC = 0.245

Table S18 Relationship between perceived facial attractiveness and facial coloration measured by spectrophotometer before vaccination

Note number of targets = 20; facial coloration measurements for one participant are missing

Model information	
Estimate	Linear mixed model fit by REML
Call	rating_atr ~ 1 + Left cheek lightness + Forehead lightness + Left cheek redness + Forehead redness + Left cheek yellowness + Forehead yellowness+(1 | rater)+(1 | target)
AIC	2922.355
BIC	2986.196
LogLikel.	-1459.210
R-squared Marginal	0.069
R-squared Conditional	0.568
Converged	yes
Optimizer	bobyqa

				95% Confidence Interval				
Predictors	**F**	**β**	**SE**	**Lower**	**Upper**	**df**	**t**	**p**
(Intercept)		3.123	0.245	2.642	3.603	15.300	12.733	< .001
Left cheek lightness	1.116	0.219	0.207	-0.187	0.625	13.000	1.056	0.310
Forehead lightness	0.391	-0.119	0.191	-0.493	0.255	13.000	-0.625	0.543
Left cheek redness	2.516	0.184	0.116	-0.043	0.412	13.000	1.586	0.137
Forehead redness	0.720	-0.116	0.137	-0.385	0.152	13.100	-0.849	0.411
Left cheek yellowness	0.746	-0.170	0.196	-0.554	0.215	13.100	-0.864	0.403
Forehead yellowness	0.611	0.176	0.226	-0.266	0.619	13.000	0.782	0.448

Random components

Groups	**Name**	**SD**	**Variance**	**ICC**
rater	(Intercept)	0.641	0.411	0.242
target	(Intercept)	1.039	1.079	0.455
Residual		1.136	1.29	

Note. Number of Obs: 878 . groups: rater 88. target 20

Table S19 Relationship between perceived facial healthiness and facial coloration measured by spectrophotometer before vaccination

Note number of targets = 20; facial colouration measurements for one participant are missing

Model information

Estimate	Linear mixed model fit by REML
Call	rating_healthiness ~ 1 + Left cheek lightness + Forehead lightness + Left cheek redness + Forehead redness + Left cheek yellowness + Forehead yellowness+(1 | rater)+(1 | target)
AIC	2298.078
BIC	2359.659
LogLikel.	-1147.338
R-squared Marginal	0.065
R-squared Conditional	0.526
Converged	yes
Optimizer	bobyqa

Predictors	F	β	SE	95% Confidence Interval Lower	95% Confidence Interval Upper	df	t	p
(Intercept)		4.408	0.240	3.938	4.879	16.600	18.364	< .001
Left cheek lightness	1.743	0.262	0.199	-0.127	0.651	13.000	1.320	0.209
Forehead lightness	1.372	-0.215	0.184	-0.575	0.145	13.200	-1.171	0.262
Left cheek redness	0.607	0.087	0.111	-0.131	0.305	13.000	0.779	0.450
Forehead redness	0.837	-0.120	0.131	-0.377	0.137	13.100	-0.915	0.377
Left cheek yellowness	2.837	-0.318	0.189	-0.687	0.052	13.200	-1.684	0.116
Forehead yellowness	1.483	0.264	0.216	-0.161	0.688	13.100	1.218	0.245

Random components

Groups	Name	SD	Variance	ICC
rater	(Intercept)	0.667	0.445	0.234
target	(Intercept)	0.986	0.973	0.400
Residual		1.207	1.456	

Note. Number of Obs: 664 . groups: rater 66. target 20

Table S20 Relationship between perceived facial attractiveness and facial coloration measured by spectrophotometer after vaccination

Note number of targets = 20; facial coloration measurements for one participant are missing

Model information	
Estimate	Linear mixed model fit by REML
Call	rating_atr ~ 1 + Left cheek lightness + Forehead lightness + Left cheek redness + Forehead redness + Left cheek yellowness + Forehead yellowness+(1 | rater)+(1 | target)
AIC	2895.224
BIC	2959.653
LogLikel.	-1445.927
R-squared Marginal	0.144
R-squared Conditional	0.552
Converged	yes
Optimizer	bobyqa

				95% Confidence Interval				
Predictors	**F**	**β**	**SE**	**Lower**	**Upper**	**df**	**t**	**p**
(Intercept)		2.939	0.213	2.523	3.356	15.500	13.832	< .001
Left cheek lightness	0.008	0.015	0.162	-0.302	0.332	12.900	0.093	0.927
Forehead lightness	0.542	0.098	0.133	-0.163	0.358	13.000	0.735	0.475
Left cheek redness	1.767	0.151	0.114	-0.072	0.374	13.000	1.329	0.207
Forehead redness	3.115	-0.206	0.117	-0.435	0.022	12.900	-1.764	0.101
Left cheek yellowness	3.896	0.405	0.205	0.002	0.807	13.000	1.974	0.070
Forehead yellowness	3.396	-0.399	0.217	-0.824	0.025	13.100	-1.843	0.088

Random components

Groups	Name	SD	Variance	ICC
rater	(Intercept)	0.596	0.355	0.220
target	(Intercept)	0.891	0.794	0.386
Residual		1.123	1.261	

Note. Number of Obs: 880 . groups: rater 88. target 20

Table S21 Relationship between perceived facial healthiness and facial coloration measured by spectrophotometer after vaccination

Note number of targets = 20; facial coloration measurements for one participant are missing

Model information

Estimate	Linear mixed model fit by REML
Call	rating_healthiness ~ 1 + Left cheek lightness + Forehead lightness + Left cheek redness + Forehead redness + Left cheek yellowness + Forehead yellowness+(1 | rater)+(1 | target)
AIC	2302.780
BIC	2364.332
LogLikel.	-1149.766
R-squared Marginal	0.081
R-squared Conditional	0.463
Converged	yes
Optimizer	bobyqa

				95% Confidence Interval				
Predictors	**F**	**β**	**SE**	**Lower**	**Upper**	**df**	**t**	**p**
(Intercept)		4.195	0.215	3.774	4.616	17.100	19.519	< .001
Left cheek lightness	0.005	-0.011	0.160	-0.324	0.302	13.100	-0.071	0.944
Forehead lightness	0.171	-0.054	0.131	-0.312	0.203	13.100	-0.414	0.686
Left cheek redness	0.883	0.105	0.112	-0.114	0.325	13.000	0.939	0.364
Forehead redness	3.264	-0.208	0.115	-0.434	0.017	13.000	-1.806	0.094
Left cheek yellowness	1.650	0.261	0.203	-0.137	0.658	13.200	1.284	0.221
Forehead yellowness	1.527	-0.263	0.213	-0.680	0.154	13.000	-1.236	0.238

Random components

Groups	Name	SD	Variance	ICC
rater	(Intercept)	0.627	0.393	0.197
target	(Intercept)	0.865	0.749	0.319
Residual		1.265	1.601	

Note. Number of Obs: 652 . groups: rater 66. target 20

CHAPTER 8

WHAT ARE YOU LOOKING AT? CONTEXT AND SEX DEPENDENCE OF VISUAL ATTENTION TO FACES

What are you looking at? Context and sex dependence of visual attention to faces

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Abstract:	Perception studies describe numerous discrete morphological facial features as important to judgments of various characteristics. Interestingly, little is known about whether people actually direct their visual attention to these features and how specific contexts or sex affect this attention. We, therefore, examined visual attention to faces in the context of intersexual (opposite-sex assessment of attractiveness) and intrasexual (same-sex assessment of dominance) selection. In total, 93 women and 33 men rated 80 high-resolution facial photographs of men and women while their gaze was recorded using eye-tracking. To explore patterns of raters' attention to faces and specific facial features, we used the number of fixations, fixation duration, and visit duration as visual attention measures. During both tasks, women directed more visual attention to faces than men (more fixations, longer visit duration) and directed more visual attention towards the faces of potential partners (more fixations, longer visit duration) than potential rivals. Facial features that acquired the

	most visual attention across contexts and sexes were the eyes, nose, and mouth, but small differences between sexes and contexts in visual attention were found for other facial regions suggested by previous perception studies, such as the chin and the cheeks indicating their importance in specific judgements.

What are you looking at? Context and sex dependence of visual attention to faces

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Abstract

Perception studies describe numerous discrete morphological facial features as important to judgments of various characteristics. Interestingly, little is known about whether people actually direct their visual attention to these features and how specific contexts or sex affect this attention. We, therefore, examined visual attention to faces in the context of intersexual (opposite-sex assessment of attractiveness) and intrasexual (same-sex assessment of dominance) selection.

In total, 93 women and 33 men rated 80 high-resolution facial photographs of men and women while their gaze was recorded using eye-tracking. To explore patterns of raters' attention to faces and specific facial features, we used the number of fixations, fixation duration, and visit duration as visual attention measures.

During both tasks, women directed more visual attention to faces than men (more fixations, longer visit duration) and directed more visual attention towards the faces of potential partners (more fixations, longer visit duration) than potential rivals. Facial features that acquired the most visual attention across contexts and sexes were the eyes, nose, and mouth, but small differences between sexes and contexts in visual attention were found for other facial regions suggested by previous perception studies, such as the chin and the cheeks indicating their importance in specific judgements.

Keywords

Mate choice; intrasexual selection; intersexual selection; attractiveness; dominance; competition

1 Introduction

People are exceptionally attentive to the faces of others (Gillath et al., 2017; Hewig et al., 2008) and spontaneously attribute many characteristics, for instance age, sex, personality, attractiveness, or dominance (Calder et al., 2011; Little, 2014; Perrett et al., 1998) based on facial appearance. These assessments are usually formed rapidly and with just thin slices of available information, such as the development of certain facial features.

The ability to adequately assess the characteristics of others based on their appearance may be crucial for making decisions about own future actions. In the context of mate choice, such decisions might be about the suitability of a potential partner (Thornhill & Gangestad, 1999, 2006). In the context of competition for mates, it can include a decision about whether one should compete with a potential rival or withdraw (Sell et al., 2012). One may therefore expect selection for neurocognitive mechanisms that facilitate adequate perception, judgment, and behaviour (Galperin et al., 2013).

Intersexual and intrasexual selection are considered to be significant selective pressures which led to the development of certain sexually dimorphic morphological traits in humans (Třebický et al., 2012). It has been suggested that in the context of intersexual selection, the attractiveness of certain traits functions as a cue to the individual's mating quality, such as health, good immune system, or developmental stability (Stephen & Luoto, 2023, but see Cai et al., 2019; Foo et al., 2017; Pátková et al., 2022). The tendency to be attracted by individuals who have attractive traits is believed to increase own fitness via direct or indirect benefits rising from potential mating with such individuals (Thornhill & Gangestad, 1999, 2006). In women, large eyes, small noses and chins, and puffy cheeks are facial traits considered attractive (Cunningham, 1986; Little, 2014), while in men, it is large and deep-set eyes and prominent cheekbones and chin (Cunningham et al., 1990; Little et al., 2011). Analogically, intrasexual selection in men is thought to shape morphological traits connected to both perceived and actual formidability, aggressiveness, dominance, or other traits related to success in competition in general (Barber, 1995; Puts, 2010). It has been found that individuals perceived as more dominant and competent in contest have more developed masculine features, such as wider cheekbones, prominent brow ridge, robust jawline, and narrow lips (Scott et al., 2013; Třebický et al., 2013; Vernon et al., 2014). Although it has been suggested that certain facial features are linked to the perception of attractiveness and dominance, it has not yet been directly investigated whether individuals actually selectively focus on these features when assessing faces or whether, alternatively, individuals perceive faces more holistically regardless of the context.

Eye-tracking is a frequently used method which provides an insight into autonomous visual attention processes. It also enables researchers to avoid potential bias connected with self-reports, which can be affected by participants' beliefs, including social desirability. Using eye-tracking, we can identify the direction of visual attention through delineated areas of interest (AOI), the number and duration of fixations (areas where the gaze rest), saccades (quick eye movements from one visual target to another), dwell time in the AOI (time spent looking at the area) or, analogously, visit duration, which is comprised of all visits in the AOI (time between the first fixation on the AOI and the next fixation outside of the AOI) including saccadic duration between those fixations. Various studies suggest that these metrics refer to different but sometimes also overlapping aspects of cognitive processes behind the visual attention (Althoff & Cohen, 1999; Duchowski, 2017; Skaramagkas et al., 2023). It has been proposed that the number of fixations could reflect the importance of areas where the gaze is directed (more important = more fixations) (Jacob & Karn, 2003), correspond to the informativeness of visual stimuli and their liking, or indicate cognitive load (Duchowski, 2017; Skaramagkas et al., 2023). The duration of fixation is mostly believed to be positively associated with task difficulty (Galley et al., 2015), while visit duration has been linked to the importance and informativeness of the visual stimulus or liking of the stimulus as well as task difficulty, where more time might be needed to extract and interpret information (Duchowski, 2017; Jacob & Karn, 2003). Given that there can be a few long fixations or numerous short fixations, which can translate to the same visit duration, it seems that in order to achieve a deeper understanding of gaze behaviour, it would be reasonable to examine the number and duration of particular fixations alongside visit duration. Finally, it is important to note that since AOIs are delineated based on the researcher's interest, studies tend to differ in their number, placement, and even shape and size, which can inherently affect the results and make their results difficult to compare (Hessels et al., 2016).

Previous studies using eye-tracking have provided information about the regions of interest when looking at people. It has been shown that both heterosexual men and women are most interested (measured in viewing duration) in the faces of opposite-sex individuals (Hewig et al., 2008). Further, heterosexuals look longer and more often at faces of the opposite sex who are potential partners than at the faces of potential friends (Gillath et al., 2017), which supports the notion of the face being especially salient in the mating context.

Eye-tracking studies have also investigated the specific facial regions in which people are generally interested. When freely looking at faces without a specific task (free-viewing paradigm), the eyes seem to draw the most visual attention, followed by either the mouth or the nose in the second place (Hickman et al., 2010; Król & Król, 2019; Semmelmann & Weigelt, 2018). Similarly, during the face recognition task, participants fixated the longest on the eyes, nose, mouth, and cheeks (Chelnokova & Laeng, 2011). Several studies examined possible differences in visual attention to facial regions in the context of specific judgements. During facial attractiveness judgements of women's faces, both men and women looked the longest at the nose and then, for similarly long times, at the eyes and the lips (Zhang et al., 2017). In that study, researchers found no sex difference in visual attention to facial features during judgements of female attractiveness, but a major limitation of this study was that the three abovementioned regions were the only AOI analysed. In other studies, which took into account a wider array of AOIs, no differences in visual attention were found when judging the age and attractiveness of the face: in both cases, participants fixated primarily on the eyes and nose (Kwart et al., 2012). Analogously, visual attention was similar during trustworthiness and dominance judgements, with the eyes, the nose, and the mouth attracting the greatest amount of visual attention (Hermens et al., 2018).

All in all, although mate choice and competition are considered to function as significant selective pressures linked to the development of specific facial features, little is known about context-dependent differences in visual attention to faces and attention to specific facial regions. While some eye-tracking studies have tested changes in visual attention to faces between different contexts, to the best of our knowledge, differences between the context of mate choice and competition have not been studied yet. Moreover, the size of the stimuli in previous eye-tracking studies varied and where the stimuli were relatively small, it may have hindered the accuracy of the measurements due to foveal vision. On top of that, although perception studies have identified several features of importance to attractiveness and dominance judgements, these features are not always specified as AOIs in the eye-tracking studies.

In the following, we therefore investigate potential differences in visual attention to faces and their individual features in the context of mate choice (opposite-sex assessment of potential partners' attractiveness) and competition (same-sex assessment of potential rivals' dominance) using eye-tracking methods. To do that, we use stimuli as close to life-size as possible, of high-quality, and with specified AIOs covering those facial features which perception studies identified as important for attractiveness and dominance judgements of male and female faces. We also examine possible effects of raters' sex. As measures of visual attention, we used the number of fixations, fixation duration, visit duration, and the AOI of first fixation. These eye movement metrics can indicate the importance or informativeness of the stimulus or its parts, and correspond to the cognitive load posed by the stimulus and the task at hand.

We expected that men and women would pay more visual attention to potential partners than to potential rivals. For between-sex differences, we hypothesised that women would pay more visual attention to faces in both contexts than men would. Regardless of the context, we anticipated that men and women would direct most of their visual attention to the eyes, the nose, and the mouth. Further, we expected that both men and women would look more at features identified by perception studies as important in dominance judgements (e.g., chin) when assessing potential rivals than when assessing potential partners and that men would direct their attention to these features more than women. Finally, we predicted that when assessing potential partners, the first fixation in both men and women would land mostly on the eyes and the mouth, while when assessing a potential rival, the first fixation would be directed at the eyes and the chin.

2 Materials and Methods

The authors assert that all procedures contributing to this work comply with the ethical standards of the national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study and its methods were approved by the IRB at Charles University (approval no. 2019/20).

Before entering the study, all participants were informed about its goals and expressed their consent with participation by signing an informed consent form. Data used in this study are part of a larger longitudinal project investigating intra- and interindividual differences in visual attention to facial features which are believed to have developed under the influence of intrasexual and intersexual selection.

2.1 Procedure

Raters, in randomised order, assessed sets of facial photographs of same-sex individuals (40) for their dominance, and facial photographs of opposite-sex individuals (40) for their attractiveness on 7-point scales based on a situation induced by a short vignette (potential partner or rival). At the same time, their eye movements were recorded by eye-tracking. Participants then completed a set of questionnaires (e.g., basic demographical data).

2.2 Raters

Raters were recruited via social media sites (Facebook), oral invitations, and posters in the halls of the Faculty of Science, Faculty of Humanities, and the Faculty of Physical Education and Sports (all Charles University, Prague, Czechia). Requirements for participation were: age 18–40 years, heterosexual, with normal or corrected-to-normal vision, and, in women, not being a user of hormonal contraception to avoid possible effect of hormonal contraception on their perception (Little et al., 2013). In total, 110 women and 35 men participated in the study. We excluded from further analyses 14 non-heterosexual women and 2 non-heterosexual men (defined as 3 and above on a 7-point scale ranging from 1–exclusively heterosexual to 7–exclusively homosexual). Further, data from three women were excluded due to insufficient quality of the eye-tracking data (where the eye-tracker did not identify the eyes correctly and fixations were either missing or on the side of the screen for most of the viewing session). The resulting sample thus consisted of 93 women (M = 23.5 ys, SD = 4.37, age range = 18–38) and 33 men (M = 23.9 ys, SD = 4.69, age range = 18–37). All raters received a reimbursement of 100 CZK (app. 4 EUR) as compensation for their time (app. 60 minutes).

2.3 Stimuli

The stimuli consisted of 80 standardised facial photographs of Czech men (40) and women (40) aged 19–34 years (men: M = 24.4 ys, SD = 4.10, women: M = 23.3 ys, SD = 4.25), a subset of photographs obtained in previous studies (Kleisner et al., 2019). We intended to keep the rating reasonably long and not too demanding for the participants. Photographs were selected based on their degree of standardisation. Targets were positioned 0.5m from plain grey background and photographed from a distance of 1.5m. They wore black t-shirts provided by researchers, assumed a neutral facial expression and refrained from any adornments such as glasses, jewellery, or makeup. Stimuli were captured with a Canon 6D full-frame DSLR equipped with an 85mm fixed focal length lens under conditions standardised in terms of targets' distance from the camera, environment, and exposure. For further details of the photo acquisition procedure, see Kleisner et al. (2019) and Třebický et al. (2018).

2.3.1 The post-processing of photographs

We used the Adobe Lightroom CC 2019 and Adobe Photoshop CC 2019 for the post-processing of photographs we had obtained. Images were colour calibrated with DNG colour calibration profiles (using the X-Rite Color Checker Passport Lightroom plugin). Evenness of exposure was manually checked and, where necessary, adjusted on the 85% value of every channel in the RGB colour space. Each participant's horizontal and vertical position within the image frame was adjusted so that the target's head was positioned in the centre of the frame with pupils on one horizontal line. Then we batch-cropped the photographs to optimally fit the heads on a 16:9 27" monitor (resulting in head size slightly smaller than real life) while preserving the relative difference in size between individuals. In the next step, a blur vignette was applied over the photos so that the face, hair, and neck remained in focus, and mainly the t-shirts and surrounding parts of the background were slightly blurred to minimise any possibly disturbing creases or shadows. Then we converted the resulting images into an sRGB colour space and exported them into an 8-bit JPEG format (1215×2160 resolution, 300 PPI, sRGB).

2.4 Eye-tracking

Rating was conducted using Tobii Studio software v 3.4.8 on a desktop computer with a 27" LCD screen (BenQ PD2700U IPS; 3840×2160, 99% sRGB colour space coverage) in the landscape position. Eye-tracker Tobii X2-60 (60Hz) was mounted to the bottom frame of the LCD monitor using a clamp and an extension arm. The eye-tracker was at a distance of 28cm in front of the screen, tilted 13° upwards towards the participant and centred to the middle of the screen. The active width and height of the LCD screen to track were set to 60×34cm, respectively. The upper edge of the eye-tracker was 4.5cm above the lower edge of the LCD monitor.

2.5 Rating

Rating took place in a quiet windowless room under standardised conditions with artificial lighting so as to eliminate any changes in ambient light. The raters sat app. 90cm from the screen with eyes at the height of 116cm (i.e., at the same eye level as the stimuli on the screen when measured from the floor to the outer corner of the eye). Raters were seated on an office chair without wheels, with an adjustable headrest and armrests. Their head was resting against the headrest and arms against armrests, which were adjusted according to their needs. A large plastic pad was positioned in their lap: on the pad, they used a mouse to carry out the rating. Next, we performed a calibration of the eye-tracker using a standard 9-point calibration scheme in the Tobii software (Blais et al., 2008). If necessary, calibration was repeated. As soon as successful calibration was achieved, raters were instructed not to move or talk unless necessary. Then they carried out one testing round to familiarise themselves with the rating interface. During this trial, a smiley was shown instead of a facial photograph but other elements were the same as in the actual rating.

Each rater assessed both male and female sets of facial photographs, each containing 40 images. The two sets and photographs in them were presented in a randomised order. Participants assessed photographs of same-sex individuals regarding their dominance and photographs opposite-sex individuals regarding their attractiveness on 7-point scales. Before they started rating a set, we induced the context of potential partner or potential rival assessment by a short vignette (Csajbók et al., 2022). It was displayed in Czech on the screen. The vignettes had the following form (for men): 'Imagine you are at a party. Suddenly, you notice that a woman standing nearby is looking at you with interest. How attractive is this woman according to you?' or 'Imagine you meet a woman at a party and spend a better part of the evening with her. Now, another man, who seemed interested in her as well, approaches her. How dominant (i.e., how capable of enticing her away) do you think the man is?' Analogous texts were displayed to women. Then a fixation cross was displayed for 1,000ms in different quadrants of the screen (never in the centre of the screen, to avoid AOI fixation bias for the area where the stimuli were about to be presented) before each facial photograph and raters were instructed to always look at the fixation cross. This was followed by a 5,000ms long presentation of the facial photograph. In the next step, a 7-point verbally anchored rating scale of attractiveness/dominance was displayed for 7,000ms on a new screen, where participants indicated their rating by clicking on the appropriate number.

After the rating session, raters completed questionnaires regarding their basic and demographic data (age, education, occupation etc.), sexual orientation, in case of women also the phase of menstrual cycle, and other questionnaires unrelated to the current investigation.

The duration of viewing each facial photograph was set to 5,000ms in the Tobii Studio software, and 5,000ms filter was also set in jamovi for the visit duration. Aside from that, if a rater recognised the depicted in the photograph, that combination of rater and stimulus was removed from analyses (five raters in total recognised a minimum of two and maximum of five people in the dataset).

2.6 AOI delineation

In comparison to some previous studies which defined as AOIs only the eyes, the nose, and the mouth (Zhang et al., 2017), we defined other areas identified by perception studies as relevant for attractiveness and dominance judgements (Cunningham, 1986; Cunningham et al., 1990; Třebický et al., 2013), such as the cheeks, chin, and the forehead, similarly to Chelnokova and Laeng (2011). Using Tobii Studio software v 3.4.8, we have thus defined the following AOIs:

right eye, left eye, nose, mouth, forehead (including hair), chin, right cheek, left cheek, right ear, and left ear manually for each stimulus. For an example of the defined AOIs, see Figure 1.

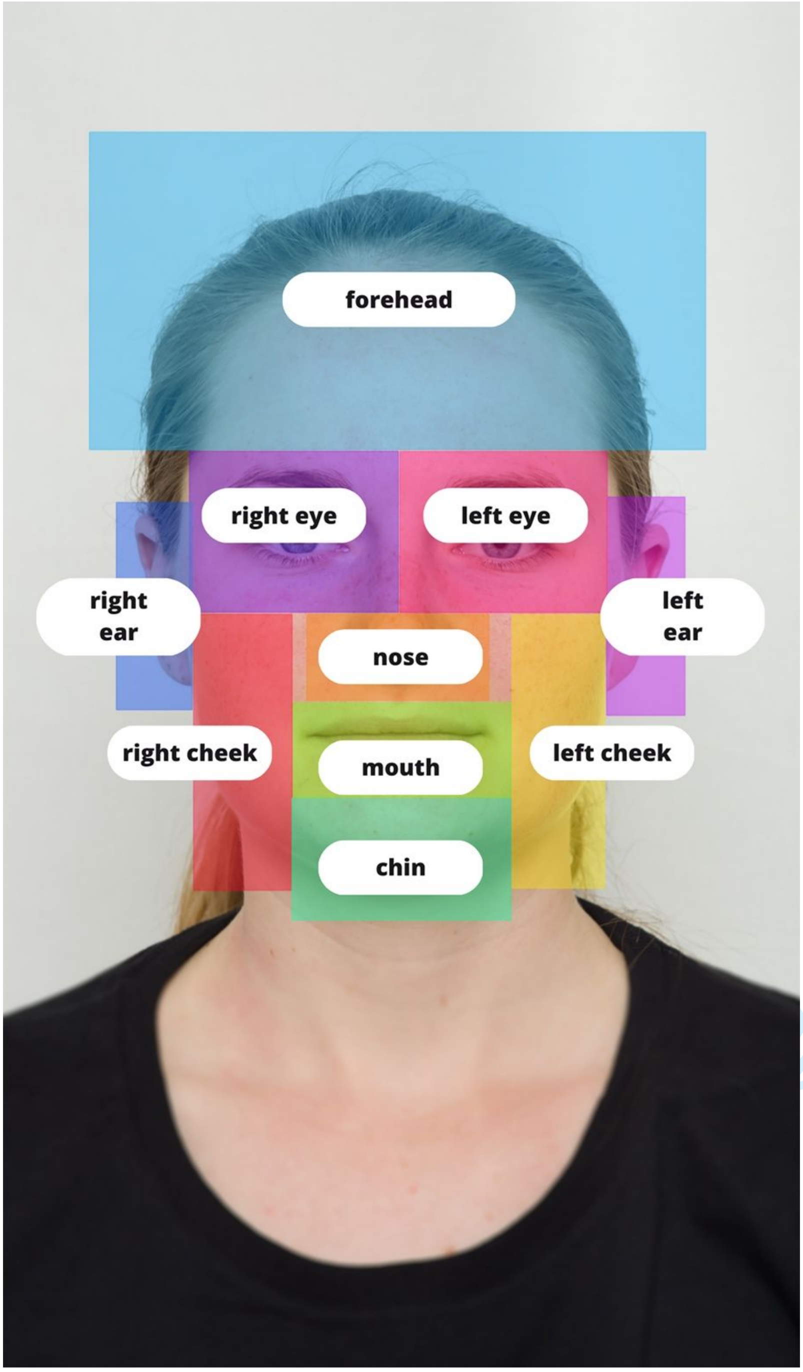

Fig. 1: Example of stimuli with delineated AOIs (informed consent was obtained to publish the image).

3 Data analyses

All statistical analyses were performed in jamovi v 2.3.21.0. Inspection of the data parameters, normality tests (Kolmogorov–Smirnov), and visual representation indicated that the data for fixation duration, visit duration, and the number of fixations on the AOIs do not follow a normal distribution but a negative binomial. Only the number of fixations on a whole face was normally distributed. To investigate the data which followed a negative binominal distribution, we employed generalised mixed-effects models, while for the normally distributed data, we used linear mixed-effects model using the GAMLj module (v 2.6.6) in jamovi.

To test the effect of context on visual attention (dominance vs. attractiveness ratings), we conducted both whole-face analyses and analyses for separate AOIs within the raters' sex. We entered the number of fixations, mean fixation duration (ms), and visit duration (ms) into separate models as dependent variables. The context of rating the attractiveness of potential partner/dominance of potential rival (attractiveness/dominance, abbreviated as atr/dom), and in the case of AOI analyses also ID AOI were entered as fixed-effect predictors. To control for the variability of targets and raters, we entered the targets' and raters' IDs as random effects. This showed that the target ID had virtually no variance, leading to a singular fit. Therefore, we report all analyses without target ID as a random effect.

Example of a model entry for whole-face analysis: N fixations per face ~ 1 + atr/dom + 1 (1|ID_rater), and AOI analyses: N fixations per AOI ~ 1+ atr/dom+ ID AOI + atr/dom:ID AOI + 1 (1|ID_rater). We performed analogous analyses for between-sex differences, which analysed each context (atr/dom) separately. To test differences between pairs of predictor levels, we used a posthoc test with Holm correction for multiple comparisons. For linear mixed-effects models, we report the proportion of variance explained by the fixed effects without random effects with R^2_M, the proportion of variance explained by both the fixed and random effects with R^2_C, and the effect size using β with 95% CI. For generalised mixed-effects models, we report fixed-effect omnibus test results with X^2 and the effect size using β and (exp)B with 95% CI.

To identify the AOI of the first fixation, we used the Chi-square test of association. For between-contexts differences, we specified AOIs as rows and context (atr/dom) as columns. For between-sex differences, we specified AOIs as rows and rater sex as columns. We report χ^2 and the strength of association with Cramer's V.

4 Results

4.1 Context-dependent differences in visual attention in women and men

4.1.1 Whole face analyses

The linear mixed-effects model showed that in women, the *number of fixations* on the face was statistically significantly predicted by the context, that is, by whether the rater was assessing a potential rival or a potential partner ($R^2_C = 0.400$, $R^2_M = 0.003$, $F (1, 7334) = 37.2$, $\beta = -0.324$, [-0.428, -0.220], $p < 0.001$). Women made, on average, more fixations (14.3 compared to 14) when assessing the attractiveness of a potential partner than when assessing the dominance of a potential rival. In men, however, the number of fixations on the face was not predicted by the context ($R^2_C = 0.402$, $R^2_M < 0.001$, $F (1, 2606) = 0.032$, $\beta = -0.017$, [-0.208, 0.173], $p = 0.858$). For details, see Figure 2 and Supplementary Materials S1 and S1J.

A generalised mixed-effects model showed that in women, the *mean fixation duration* was not statistically significantly predicted by the context, i.e., assessment of potential rival vs. potential partner ($X^2 (1) = 0.818$, $\beta = 0.005$, exp(B) = 1, 95% exp(B) CI [0.995, 1.01], $p = 0.366$). In men, the model showed that the mean fixation duration was statistically significantly predicted by the context ($X^2 (1) = 11.7$, $\beta = -0.033$, exp(B) = 0.967, [0.949, 0.986], p = < 0.001). Specifically, we found that in men, on average, the duration of fixation is longer when assessing potential partners compared to rivals (276ms compared to 267ms). For details, see Figure 2 and Supplementary Materials S1 and S1J.

A generalised mixed-effects model showed that in women, face *visit duration* (time spent at whole face) was predicted by the context ($X^2 (1) = 7.24$, $\beta = -0.012$, exp(B) = 0.988, 95% exp(B) CI [0.980, 0.997], $p = 0.007$) who had, on average, longer visit duration on the face when assessing attractiveness than when assessing dominance (4,028ms vs. 3,980ms). In men, the model showed that the context did not predict visit duration on the face ($X^2 (1) = 2.49$, $\beta = -0.016$, exp(B) = 0.984, 95% Exp(B) CI [0.964, 1.00], $p = 0.115$). For details, see Figure 2 and Supplementary Materials S1 and S1J.

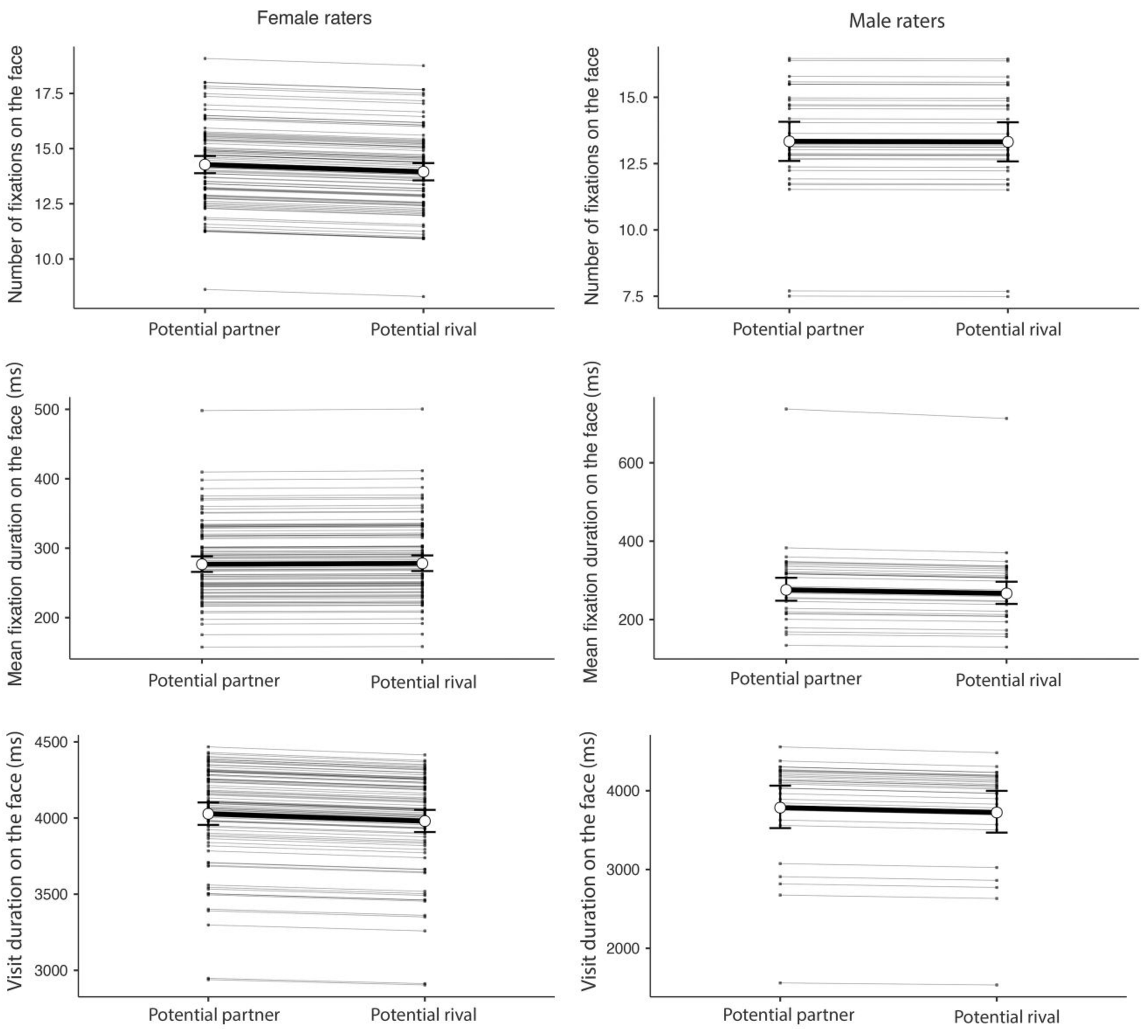

Fig. 2: Context-related differences in visual attention in male (right) and female (left) raters in whole-face analyses. From top to bottom: The number of fixations, mean fixation duration, and visit duration (in milliseconds), with female raters in the left and male raters in the right column. White dots represent mean values, error bars their 95% confidence intervals. Grey lines and dots represent random effects plotted by ID rater.

4.1.2 AOI analyses

A generalised mixed-effects model showed that in women, *the number of fixations* in AOIs was predicted by the context (X^2 (1) = 7.57, p = 0.006), by the AOI (X^2 (9) = 19498.98, p < 0.001), and by interaction between the context and the AOI (X^2 (9) = 56.32, p = < 0.001). Women made statistically significantly more fixations at AOIs when assessing potential partners than when assessing potential rivals (2.01 vs. 1.94). Regardless of the context, though, most fixations focused on average on the left (4.84) and the right eye (4.72), the nose (2.22), and the mouth (2.03). Women made, on average, more fixations on the left cheek when assessing potential partners than when assessing potential rivals (1.88 vs. 1.45). Further, women made more fixations on the right eye when assessing potential rivals than when assessing potential partners (4.8 vs. 4.63). For details, see Figure 3 and Supplementary Materials S2 and S2J.

In men, the generalised mixed-effects model showed that the number of fixations in AOIs was not predicted by the context (X^2 (1) = 3.72, p = 0.054) but was predicted by the AOI (X^2 (9) = 4261.91, p < 0.001), and there was no statistically significant interaction between the context and the AOI (X^2 (9) = 7.23, p = 0.614). Regardless of the context, men made on average the most fixations on the left eye (4.29), the right eye (3.98), the mouth (2.55), and the nose (2.51). For details, see Figure 3 and Supplementary Materials S2 and S2J.

The generalised mixed-effects model had shown that in women, *mean fixation duration* in AOIs was not predicted by the context (X^2 (1) = 0.313, p = 0.576), but was predicted by the AOI (X^2 (9) = 1855.839, p < 0.001), and there was a statistically significant interaction between the context and the AOI (X^2 (9) = 34.554, p < 0.001). Regardless of the context, women had on average the longest mean fixation durations on the left eye (304ms), the mouth (292ms), the right eye (280ms), and the left (258ms) and right (253ms) ear, followed by the nose (240ms). Moreover, women exhibited on average a statistically significantly longer mean fixation duration on the chin when assessing potential partners rather than potential rivals (244ms vs. 222ms). For details, see Figure 3 and Supplementary Materials S2 and S2J.

For men, the generalised mixed-effects model showed that the mean fixation duration on AOIs was not predicted by the context (X^2 (1) = 0.134, p = 0.714) but was predicted by the AOI (X^2 (9) = 475.692, p < 0.001), and there was an interaction between the context and the AOI (X^2 (9) = 35.062, p < 0.001). Regardless of the context, men had the longest mean fixation durations on the mouth (296ms), the left (288ms) and right eye (271ms), and the nose (251ms). Men had a statistically significantly longer mean fixation duration on the left eye when assessing potential partners rather than potential rivals (298ms vs. 279ms). For details, see Figure 3 and Supplementary Materials S2 and S2J.

The generalised mixed-effects model had shown that in women, the *visit duration* in AOIs was predicted by the context (X^2 (1) = 21.4, p = < 0.001), by the AOI (X^2 (9) = 25198.7, p < 0.001), and by interaction between the context and the AOI (X^2 (9) = 112.3, p < 0.001). Women had a significantly longer visit duration to AOIs during assessment of potential partners than when assessing potential rivals (526ms vs. 501ms). Further, regardless of the context, the longest mean visit duration was on the left (1489ms) and the right eye (1363ms), the mouth (590ms), the nose (552ms), and the forehead (487ms). Moreover, when assessing potential partners vs.

potential rivals, women spent significantly longer time (visit duration) looking at the chin (383ms vs. 297ms), the left cheek (388ms vs. 324ms), and the nose (571ms vs. 533ms), but when assessing a potential rival, they had longer visit duration on the right eye than when assessing a potential partner (1,407ms vs. 1,320ms). For details, see Figure 3 and Supplementary Material S2 and S2J.

In men, the generalised mixed-effects model showed that visit duration in AOIs was predicted by the context ($X^2 = 14.4$, $p = < 0.001$), by the AOI ($X^2 = 5519.9$, $p = < 0.001$), and by interaction between the context and the AOI ($X^2 = 35.1$, $p = < 0.001$). Men had statistically significantly longer mean visit duration in AOIs when assessing potential rivals than when assessing potential partners (513ms vs. 478ms). Regardless of the context, the longest visit durations were on the left (1,277ms) and right eye (1,140ms), the mouth (777ms), the nose (677ms), and the forehead (492ms). Lastly, men had statistically significantly longer visit duration on the chin during the assessment of potential rival than potential partner (360ms vs. 274ms). For details, see Figure 3 and Supplementary Materials S2 and S2J.

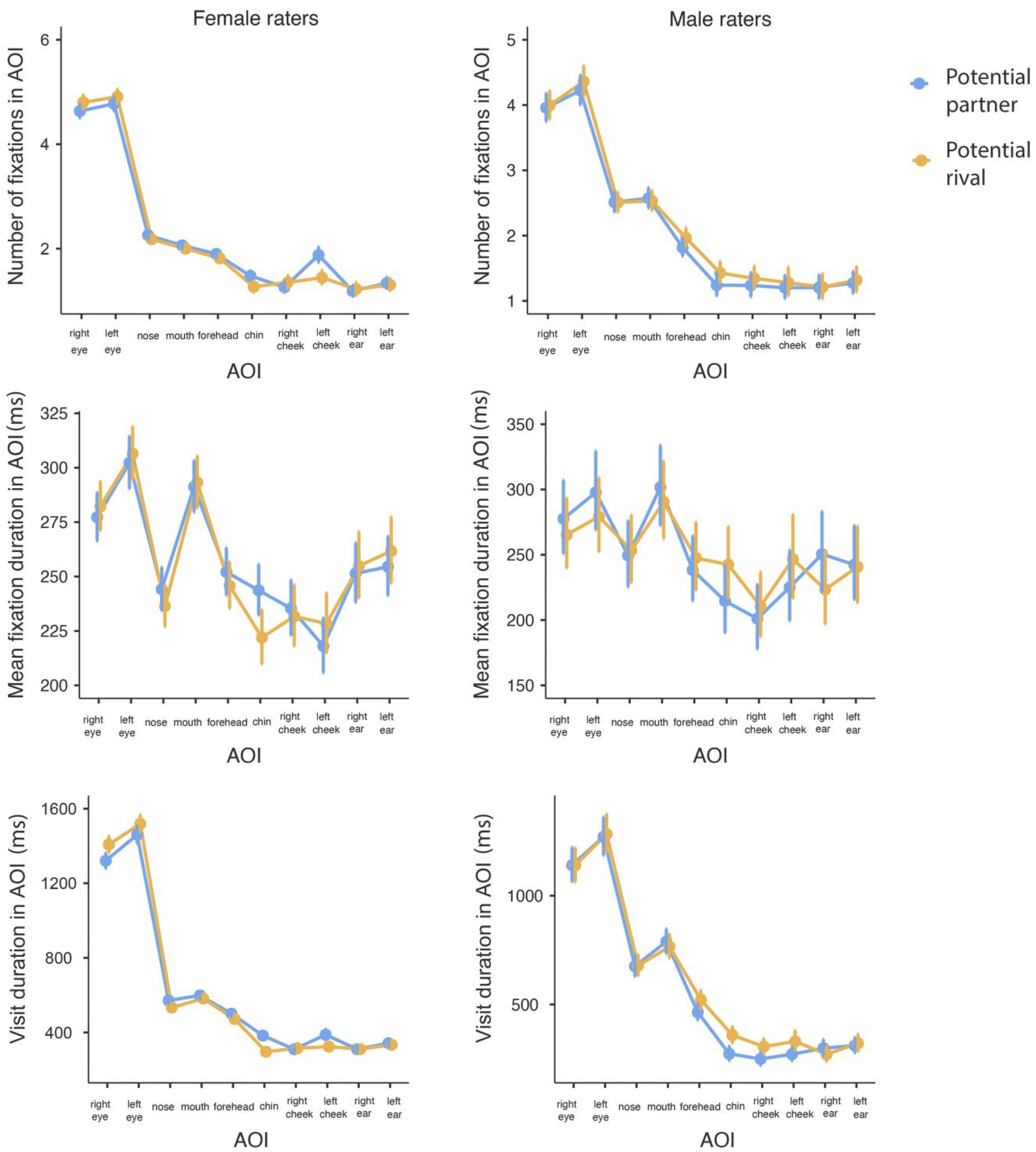

Fig. 3: Context-related differences in visual attention in males and females in AOI analyses. From top to bottom: The number of fixations, mean fixation duration, and visit duration (all with respect to particular AOIs). Female raters are on the left, male raters on the right. Dots represent mean values; error bars show their 95% confidence intervals.

In women, chi-square test showed no statistically significant difference in *the area of first fixation* between contexts: $\chi 2$ (9, N = 7396) = 6.67, p = 0.671, Cramer's V = 0.03. Regardless of the context, the areas most frequently fixated as the first were the right and the left eye, the nose, the forehead, and the mouth. For details, see Supplementary materials S3 and S3J.

In men, the chi-square test showed a statistically significant difference in the area of the first fixation between contexts: $\chi 2$ (9, N = 2628) = 27.3, p = 0.001, Cramer's V = 0.102. Regardless of the context, the areas most frequently fixated as the first were the right and the left eye, the nose, the mouth, and the forehead. When rating a potential partner, first fixation was directed more frequently than expected (if it were by chance) to the right eye, the nose, the forehead, and the right and left cheek than during rating of a potential rival. When rating a potential rival, men fixated first more often than expected on the left eye, the mouth, and the chin than during rating of a potential partner. For details, see Supplementary materials S3 and S3J.

4.2 Sex differences in visual attention in different contexts

4.2.1 Whole face analyses

The linear mixed-effects model showed that the *number of fixations* on the face during the assessment of potential partners (attractiveness) was predicted by the rater's sex (R^2_C = 0.468, R^2_M = 0.019, F (1, 124) = 5.20, β= -0.937, [-1.74, -0.132], p = 0.024). Women made statistically significantly more fixations on the whole face when assessing the attractiveness of potential partners than men did (on average 14.3 fixations vs. 13.3). For assessment of potential rivals (dominance), the linear mixed-effects model showed no difference between men and women in the number of fixations on the face (R^2_C = 0.419, R^2_M = 0.008, F (1, 124) = 2.36, β= -0.631, [-1.44, 0.174], p = 0.127). For details, see Figure 4 and Supplementary Materials S4 and S4J.

The generalised mixed-effects model showed that the *mean fixation duration* on the whole face was predicted by the rater's sex neither in the context of rating potential partners (attractiveness) (X^2 (1) = 0.013 β = -0.005, exp(B) = 0.995, 95% exp(B) CI [0.904, 1.00], p = 0.911) nor in the context of rival assessment (dominance) (X^2 (1) = 0.819, β = -0.044, exp(B) = 0.957, 95% exp(B) CI [0.871, 1.05], p = 0.365). For details, see Figure 4 and Supplementary Materials S4 and S4J.

The generalised mixed-effects model had shown that the *visit duration* on the whole face was predicted by rater's sex both when assessing potential partners (attractiveness) (X^2 (1) = 7.15, β = -0.064, exp(B) = 0.938, 95% exp(B) CI [0.895, 0.983], p = 0.007) and when assessing potential rivals (dominance) (X^2 (1) = 4.21, β = -0.069, exp(B) = 0.934, 95% exp(B) CI [0.874, 0.997], p = 0.040). In both contexts, women exhibited longer visit duration on the face than men did (attractiveness: avg. 4,025ms in women vs. 3,776ms in men; dominance: avg. 3,976ms in women vs. 3,712ms in men). For details, see Figure 4 and Supplementary Materials S4 and S4J.

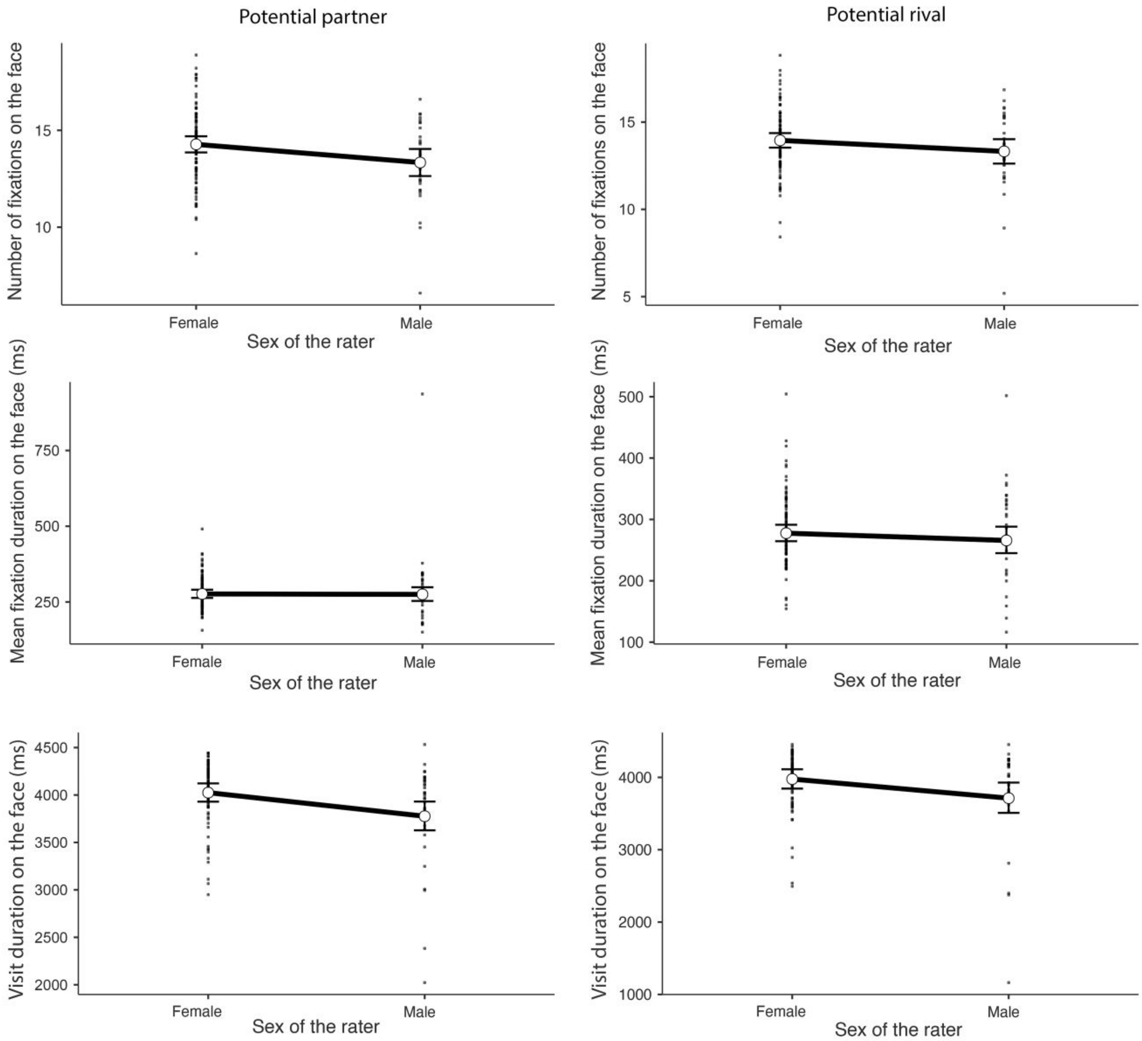

Fig. 4: Sex differences in visual attention in different contexts in whole-face analyses. From top to bottom: the number of fixations, mean fixation duration, and visit duration (in milliseconds); assessment of potential partners (attractiveness) on the left, assessment of potential rivals (dominance) on the right. White dots represent mean values, error bars show their 95% confidence intervals. Grey dots represent random effects plotted by ID rater.

4.3 AOI analyses

The generalised mixed-effects model had shown that in assessments of potential partners, the *number of fixations* in AOIs was predicted by the rater's sex ($X^2 (1) = 4.76$, $p = 0.029$), by the AOI ($X^2 (9) = 7969.68$, $p < 0.001$), and by interaction between the AOI and raters' sex ($X^2 (9) = 266.02$, $p < 0.001$). Women had, on average, statistically significantly more fixations on AOIs than men did (2.01 vs. 1.87). Regardless of rater's sex, most fixations targeted, on average, the right (4.28) and the left (4.49) eye, the nose (2.38), and the mouth (2.3). When assessing potential partners, women, compared to men, exhibited on average statistically significantly more fixations on the left cheek (1.86 vs. 1.19), the left eye (4.77 vs. 4.22), and the right eye (4.64 vs. 3.96). When assessing potential partners, men had statistically significantly more fixations on the mouth than women did (2.56 vs. 2.06). For details, see Figure 5 and Supplementary Materials S5 and S5J.

For the assessment of potential rivals, the generalised mixed-effects model shows that the number of fixations in AOIs was not predicted by the rater's sex (X^2 (1) = 0.139, p = 0.709) but was predicted by the AOI (X^2 (9) = 8664.294, p < 0.001) and by interaction between the AOI and rater's sex (X^2 (9) = 328.793, p < 0.001). Regardless of the rater's sex, most fixations were directed, on average, to the right (4.38) and left (4.62) eye, the nose (2.34), and the mouth (2.25). In assessments of potential partners, women had on average statistically significantly more fixations than men did on the left eye (4.91 vs. 4.36) and right eye (4.8 vs. 4). Men had on average statistically significantly more fixations than women on the mouth (2.53 vs. 2) and the nose (2.5 vs. 2.18). For details, see Figure 5 and Supplementary Materials S5 and S5J.

The generalised mixed-effects model had shown that when assessing potential partners, the *mean fixation duration* on AOIs was not predicted by rater's sex (X^2 (1) = 0.392, p = 0.531) but was predicted by the AOI (X^2 (9) = 931.729 p < 0.001) and by interaction between the AOI and the rater's sex (X^2 (9) = 45.721, p < 0.001). Regardless of rater's sex, the longest mean fixation duration was, on average, on the left eye (299ms), the mouth (298ms), the right eye (277ms), and the nose (246ms). There were no statistically significant differences between men and women in the mean fixation duration in the individual AOIs in comparisons of our interest. For details, see Figure 5 and Supplementary Materials S5 and S5J.

The generalised mixed-effects model shows that in assessments of potential rivals, mean fixation duration in AOIs was not predicted by the raters' sex (X^2 (1) = 0.499, p = 0.480), but was predicted by the AOI (X^2 (9) = 744.425, p < 0.001) and by interaction between the AOI and rater's sex (X^2 (9) = 88.855, p < 0.001). Regardless of the rater's sex, the longest mean fixation duration was on average on the left eye (292ms), the mouth (289ms), the right eye (273ms), the forehead (245ms), and the nose (244ms). We found no statistically significant differences between males and females in the mean fixation duration in the individual AOIs in comparisons of our interest. For details, see Figure 5 and Supplementary Materials S5 and S5J.

For assessments of potential partners, the generalised mixed-effects model shows that *visit duration* in AOIs was predicted by rater's sex (X^2 (1) = 8.18, p = 0.004), by the AOI (X^2 (9) =

10753.28, $p < 0.001$), and by interaction between the AOI and rater's sex (X^2 (9) = 360.37, $p < 0.001$). When assessing potential partners, women had on average longer visit duration in the AOIs than men did (523ms vs. 477ms). Regardless of the rater's sex, the longest visit duration was on average on the left (1,361ms) and the right (1,232ms) eye, the mouth (690ms), and the nose (618ms). When assessing potential partners, women gazed on average statistically significantly longer (visit duration) than men on the chin (383ms vs. 274ms), the left cheek (368ms vs. 268ms), the right cheek (310ms vs. 250ms), the left eye (1,462ms vs. 1,267ms), and the right eye (1,327ms vs. 1,143ms), while men gazed statistically significantly longer (visit duration) at the nose (670ms vs. 570ms) and the mouth (793ms vs. 600ms) than women did. For details, see Figure 5 and Supplementary Materials S5 and S5J.

For assessments of potential rivals, the generalised mixed-effects model shows that visit duration in AOIs was not predicted by the rater's sex (X^2 (1) = 0.242, $p = 0.623$), but was predicted by the AOI (X^2 (9) = 10438.211, $p < 0.001$) and by interaction between the rater's sex and the AOI (X^2 (9) = 397.034, $p < 0.001$). Regardless of the rater's sex, the longest visit duration was on average on the left (1,395ms) and right (1,262ms) eye, the mouth (663ms), and the nose (603ms). When assessing potential rivals, women gazed on average statistically significantly longer than men did (visit duration) on both the left (1,517ms vs. 1,284ms) and the right eye (1,398ms vs. 1,140ms). Men gazed on average statistically significantly longer than women did (visit duration) on the chin (359ms vs. 296ms), the nose (679ms vs. 535ms), and the mouth (756ms vs. 581ms). For details, see Figure 5 and Supplementary Materials S5 and S5J.

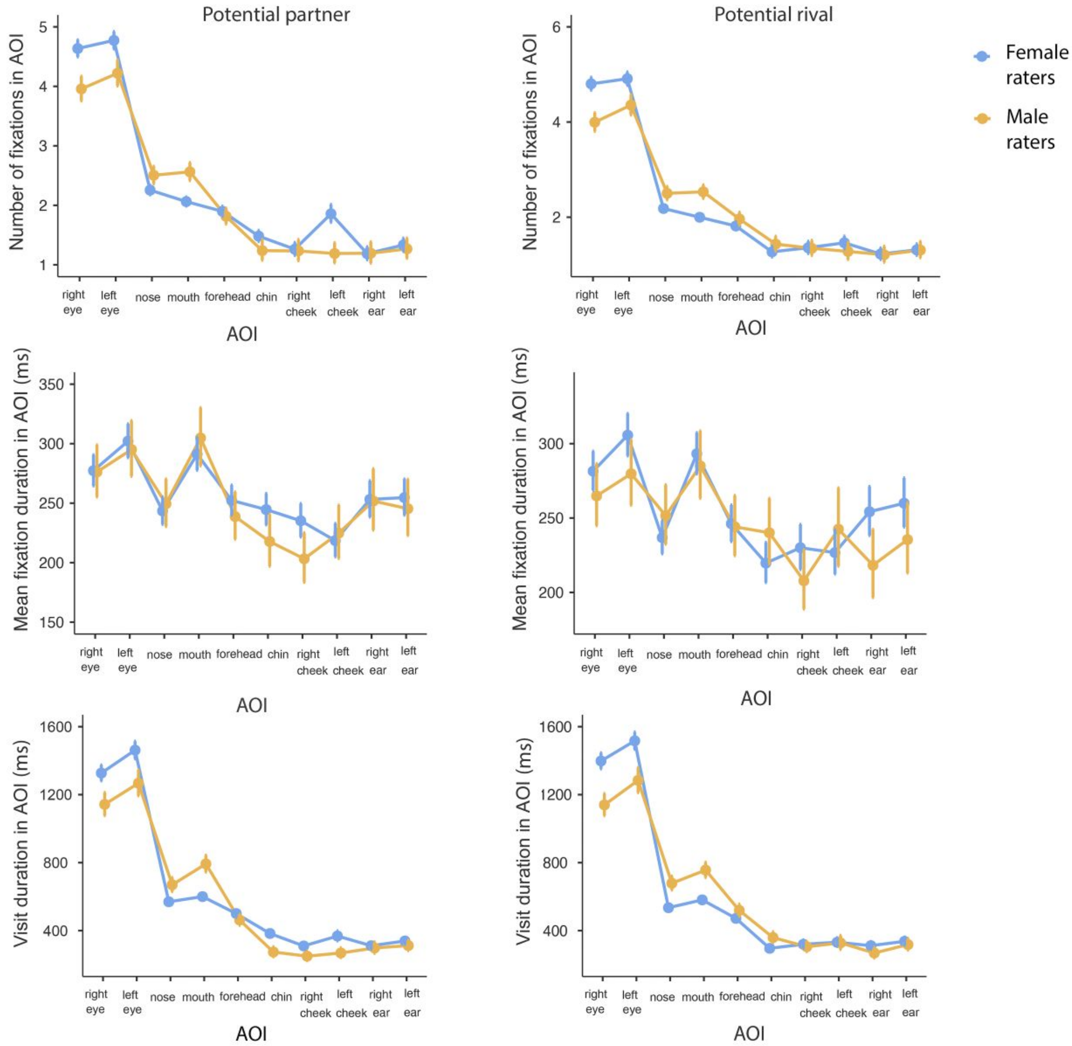

Fig. 5: Sex differences in visual attention to AOIs in different contexts. From top to bottom: the number of fixations, mean fixation duration, and visit duration in the context of assessment of potential partners (attractiveness) on the left and potential rivals (dominance) on the right. Dots represent mean values; error bars show their 95% confidence intervals.

Using the chi-square test of association, we found a significant difference between the sexes in the *area of first fixation* during assessment of potential partners: $\chi 2$ (9, N = 5017) = 65, $p < 0.001$, Cramer's V = 0.114. The area most frequently fixated as first in both sexes was the right and left eye, the nose, the forehead, and the mouth. When assessing potential partners, women's first fixation aimed more often than expected on the right and the left eye, while men's first fixation aimed more often than expected at the nose, the mouth, and the forehead. For details, see Supplementary materials S6 and S6J.

Using the chi-square test of association, we found a significant difference between the sexes in the area of the first fixation in assessments of potential rivals: $\chi 2$ (9, N = 5007) 80.1, $p < 0.001$, Cramer's V = 0.126. In both sexes, the most frequent first fixated areas were the right and the left eye, the nose, the forehead, and the mouth. In assessments of potential rivals, women's first fixation aimed more often than expected at the right eye, the left eye, the nose, and the right and left cheek, while men's first fixation aimed more often than expected at the mouth, the forehead, and the chin. For details, see Supplementary materials S6 and S6J.

5 Discussion

The main aim of this study was to explore, using a suite of eye-tracking methods, any possible differences in visual attention (the number of fixations, fixation duration, and visit duration) to faces and their features in the context of mate choice (potential partner's attractiveness rating) and competition (potential rival's dominance rating). We investigated possible between-sex differences in visual attention in the two contexts using close-to-life-size, high-quality stimuli.

When it comes to the whole face, women had marginally more fixations and longer visit duration on the face when assessing potential partners than when assessing potential rivals, while men had longer mean fixation duration when looking at faces of potential partners than face of potential rivals. To examine the importance of different facial features in the assessments, we have investigated the contribution of particular areas of interest (AOIs) identified based on previous research of facial perception. In both of the contexts described above, both men and women looked the most at the eyes, the nose, and the mouth, while the other areas (e.g., chin, cheeks) attracted little direct visual attention. Women made more fixations and had longer visit duration on the whole face than men did in both of the analysed contexts. Analyses of the AOIs revealed that the areas most looked-at by both sexes are the eyes, the nose, and the mouth. In both of the analysed contexts, women made statistically significantly more fixations and had longer visit durations on the eyes than men did, while men made more fixations and had longer visit durations on the mouth and the nose than women did. In line with our expectations, we found variations in visual attention between contexts and sexes for mouth and nose and also for features suggested by perception studies such as cheeks and chin.

Several previous studies provided some insight into contextual differences in visual attention to faces, but no direct comparison between mate choice and competition had been undertaken as yet. As noted above, we found that women made more fixations and had longer visit duration on faces when assessing potential partners than when assessing potential rivals. This suggests that women may be more interested in assessing opposite-sex potential partners rather than same-sex rivals. This is in line with the findings of Gillath and colleagues (2017), where men and women paid more visual attention to faces of potential partners than to the faces of friends, and with another study which showed that heterosexual individuals are interested in the faces of opposite-sex individuals more than in same-sex faces (Hewig et al., 2008). Further, no statistically significant differences in visual attention between the two contexts were found for men, except for a marginally longer mean fixation duration when assessing potential partners. According to Galley (2015), the lengthening of fixation duration signifies attention and cognitive control, which might be the case also in our study.

Regarding the particular facial features which attracted attention, our results are in line with previous eye-tracking studies (Hermens et al., 2018; Kwart et al., 2012) and show that, regardless of the context and rater's sex, areas which attract the most visual attention are the eyes, the nose, and the mouth. This contrasts with a number of perception studies which indicated the importance of certain other facial features for judgements of attractiveness and dominance, for instance, besides the eyes, the nose, and the lips, also the chin and cheeks (Cunningham, 1986; Cunningham et al., 1990; Scott et al., 2013) and with those studies which suggested that faces are recognised rather by parts with the need to direct one's gaze to those parts for detailed processing (Martelli et al., 2005). On the other hand, our results also should not be interpreted as implying that features other than the eyes, the nose, and the mouth play no role in attractiveness or dominance judgements at all. Eye tracking shows where the gaze is directed specifically, and although we used stimuli as close to life size as possible, it does not mean that the raters did not have the remaining features in their field of vision. In other words, although the gaze was directed at for instance the eye, other features would have been still visible and could contribute to the judgement. Moreover, the heightened interest in the eyes, the nose, and the mouth could be guided not only by interest in those facial features which are most conspicuous and/or ornamented but also by the assessment of intentions (via direction of the gaze of the target, possible vocalisation, but also recognition of emotions), which is important in the formation of the first impression and, in our evolutionary past, would have been essential for avoiding costly mistakes (Kleisner & Saribay, 2019).

Although the eyes, the nose, and the mouth attracted by far the most visual attention, we detected small but statistically significant differences between contexts in visual attention to some other facial features as well. For instance, women paid more visual attention (measured as longer visit duration) to the chin and the left cheek when assessing potential partners than when assessing potential rivals, which might point towards their importance in attractiveness judgements. This is in line with the proposed importance of the eyes, cheekbones, and chin for judgements of male attractiveness (Cunningham et al., 1990). On the other hand, the chin also seems to be salient for dominance judgements in previous morphological studies, mainly in men (Třebický et al., 2013). This is supported by our finding that men had longer visit duration on the chin when assessing potential rivals than when assessing potential partners. Therefore, while for women the chin was more important in the mating context than in rivalry, for men it was the opposite. Our results thus suggest that for men the chin plays a more important role in male intrasexual competition (Keating, 1985; Vernon et al., 2014) than mate choice. It is also possible that chin is more salient feature in male faces in general as both men and women used it (had longer visit duration) for their respective assessments.

We have observed sex differences in visual attention to faces in different contexts. When assessing potential partners, women exhibited more fixations and longer visit duration on the face than men did, which may be a sign of interest in the task or a cue to the relative importance of the task for women in comparison to men. Further, when assessing potential rivals, women exhibited longer visit duration on the face than men. Although it was not statistically significant in the separate models, it is possible that women were slightly lengthening the duration of fixations in the rival assessment task and/or had more fixations, or even longer saccades, which resulted in a marginally longer visit duration on the face. As noted above, a lengthening of the duration of fixation may signify higher cognitive load (Galley et al., 2015). It is thus possible that the task may have been more cognitively challenging and demanded more attention (hence the possibly slightly longer mean fixation duration). It is possible that women are not used to judging other women's dominance and focus on different characteristics (e.g., attractiveness). Along similar lines, it is possible that, for women, assessment of potential partners is an easier and probably also more common task.

In men, the lower number of fixations (when rating potential partners) and shorter visit duration (during both tasks) may signify that they were less interested in the tasks than women were or that neither task required increased attention because men are more used to assessing both female attractiveness and male dominance than women are. It has been proposed that contest could have been the primary mechanism of sexual selection in men (Puts, 2010), because male-to-male physical contest would have been probably more common in ancestral environments than female-to-female physical contest was (Buss, 2015). It is thus possible that men have more highly developed neurocognitive and behavioural processes facilitating both types of judgements than women do, since women generally rely mostly on indirect means of competition, such as derogation of potential rivals or gossiping (Campbell, 2004; Fisher, 2004).

In relation to sex differences in visual attention to AOIs, we observed that regardless of the task, women made more fixations and had longer visit durations in the eye region than men did. This is in line with the study by Hall and colleagues (2010), which focused on recognition of facial expression and suggested that women are better at it thanks to paying more attention to the eyes. In this study, we used facial photographs of individuals with a neutral expression but still were able to detect in women increased visual attention to the eye area. Further, we observed small but statistically significant sex differences in visual attention in the two contexts. When assessing potential partners, women exhibited more fixations and longer visit duration on the left cheek and longer visit duration on the chin and right cheek than men did. This may be due to the importance of facial features such as cheekbones and jawline in attractiveness judgements (Cunningham et al., 1990; Little et al., 2011). In contrast, men, when assessing potential partners, exhibited longer visit duration on the nose and the mouth than women did, which may indicate attention to potentially attractive and neotenous features in which the appearance of the nose and lips plays an important role (Cunningham, 1986; Keating, 1985). When assessing potential rivals, men exhibited longer visit duration on the chin, the nose, and the mouth than women did. Mouth and chin have been previously identified as important in dominance judgements (Rhodes, 2006; Scott et al., 2013); our findings thus provide further support to their relevance.

We have observed a variation in visual attention to the left and right facial features. A number of studies showed that individuals rely mostly on information contained in the left side of their visual field. This is especially pronounced in the case of faces due to the putative right-sided dominance in the brain during face processing (Ashwin et al., 2005; Bourne, 2008; Burt & Perrett, 1997). For instance, participants' judgements of gender, expression, facial attractiveness, and age were influenced more by the left side of the stimulus (from rater's perspective) and the first fixation on the face targeted the left side in most trials (Butler et al., 2005; Philips & David, 1997). Research has not, however, found any significant analogical effect for the number of fixations and fixation duration in general (Butler et al., 2005). In our study, we observed that the right eye (left-hand side from the rater's perspective) was the most often fixated first AOI in both sexes and both contexts. On the other hand, other measures of visual attention did not display a clear pattern or went even in the opposite direction: for instance, majority of visual attention (measured in the number of fixations, mean fixation duration, and visit duration) was aimed in many conditions at the left eye (raters' right-hand side), although differences between the sides were quite small. This indicates that visual processing, including the processing of faces, can be influenced by numerous factors, ranging from individual differences (Mehoudar et al., 2014) to environmental and cultural ones (such as the direction of reading) (Butler et al., 2005).

Limitations

There are several limitations to our study. One potentially limiting factor might be a disbalance in our rater sample with fewer male than female raters. Still, both our male and female sample sizes were larger or at least comparable to many similar previous eye-tracking studies (e.g., Yang et al., 2015; Zhang et al., 2017). Another limiting factor might be the relatively low mean age of both the raters and the individuals who posed as stimuli (both were mostly young university students) giving little insight into possible patterns in the general population. On the other hand, the match in age of rates and stimuli can be seen as an advantage of our sample as participants were assessing potential mates and rivals of roughly the same age.

Future directions

Future research in this area should focus on investigating what role varying degrees of attractiveness and dominance play in the visual attention towards faces. Also, methods of geometric morphometrics could be employed to clarify whether specific facial regions associated with perception of particular characteristics (e.g., attractiveness, sex-typicality) overlap with facial regions (AOIs) that attract visual attention. Lastly, studies should measure not only which features attract visual attention but whether their appearance affects the attention.

6 Conclusion

Our study contributes to research into visual attention to faces by examining it in two evolutionarily relevant contexts, namely assessment of potential partners (attractiveness rating) and potential rivals (dominance rating) and investigating sex differences in visual attention. Further, we used nearly-life-sized, high-quality stimuli and defined a wider array of theory-driven AOIs on the face than most studies do. We found contextual differences in visual attention to whole faces only in women, who exhibited more fixations and longer visit duration when assessing potential partners, which points towards the importance of the task. Regarding sex differences, both tasks may have been more engaging and important but also more demanding for women than for men since, in women, we found longer visit duration on the whole face in comparison to men in both contexts. Previous perception studies identified numerous morphological facial features as being important to judgements of attractiveness or dominance: besides the eyes, the nose, and the mouth also for instance the cheeks and the chin. Our study shows that the eyes, the nose, and the mouth are areas that indeed attract most visual attention across sexes and contexts. Nevertheless, in line with perception studies and our predictions, we have also found small differences in visual attention to the cheeks and the chin. For women, these features seem to be more important in the mating context, while for men, the chin seems to be a more salient source of information in male intrasexual competition as attested by the fact women had longer visit duration when assessing potential partners and men when assessing potential rivals, respectively. Overall, our study suggests that visual processing of faces and attention towards individual facial features is to some extent both context- and sex-dependent.

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Conflicts of interest declaration

Declarations of interest: none.

Data availability

The data and Supplementary materials associated with this research are available at https://osf.io/fgvma/?view_only=37c2f0937576474ba245f21196962cae.

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CHAPTER 9

POSSIBLE DIFFERENCES IN VISUAL ATTENTION TO FACES IN THE CONTEXT OF MATE CHOICE AND COMPETITION

Evolution and Human Behavior

Possible differences in visual attention to faces in the context of mate choice and competition

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Abstract:	Existing research indicates that the shape of various facial regions is linked to perceived attractiveness and perceived formidability. Interestingly, there is little evidence showing that people actually focus on these specific facial regions during judgements of attractiveness and formidability and little support for the notion that the levels of attractiveness and formidability affect raters' visual attention. We have employed eye tracking to examine visual attention (the number of fixations and dwell time) in 40 women and 37 men while they assessed 45 male faces in life-sized photographs. The facial photographs were grouped by varying levels of attractiveness and formidability (low, medium, high). Our results show that regardless of the characteristics rated, both men and women paid the most visual attention to the eyes, nose, mouth, and forehead regions. We found statistically discernible variation in visual attention in relation to the rater's sex or target's attractiveness levels for other facial features (the chin, cheeks, or ears), but these differences may not be substantial enough to have practical implications. We suggest that the eyes, the nose, and the mouth regions play a central role in the evolution of face perception as regions most salient to the acquisition of informative cues about others. Further, during both attractiveness and formidability judgements, men looked longer at the stimuli than women did, which may hint at increased difficulty of this task for men – perhaps because they compare themselves with the stimuli. Additionally, irrespective of sex, raters looked marginally longer at faces with a medium level of formidability than at those with a high formidability level, which may reflect ambiguity of these stimuli and uncertainty regarding assessment. We found no other relationships between the target's attractiveness and formidability level in the context of visual attention to whole faces.

Possible differences in visual attention to faces in the context of mate choice and competition

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Abstract

Existing research indicates that the shape of various facial regions is linked to perceived attractiveness and perceived formidability. Interestingly, there is little evidence showing that people actually focus on these specific facial regions during judgements of attractiveness and formidability and little support for the notion that the levels of attractiveness and formidability affect raters' visual attention.

We have employed eye tracking to examine visual attention (the number of fixations and dwell time) in 40 women and 37 men while they assessed 45 male faces in life-sized photographs. The facial photographs were grouped by varying levels of attractiveness and formidability (low, medium, high).

Our results show that regardless of the characteristics rated, both men and women paid the most visual attention to the eyes, nose, mouth, and forehead regions. We found statistically discernible variation in visual attention in relation to the rater's sex or target's attractiveness levels for other facial features (the chin, cheeks, or ears), but these differences may not be substantial enough to have practical implications. We suggest that the eyes, the nose, and the mouth regions play a central role in the evolution of face perception as regions most salient to the acquisition of informative cues about others. Further, during both attractiveness and formidability judgements, men looked longer at the stimuli than women did, which may hint at increased difficulty of this task for men – perhaps because they compare themselves with the stimuli. Additionally, irrespective of sex, raters looked marginally longer at faces with a medium level of formidability than at those with a high formidability level, which may reflect ambiguity of these stimuli and uncertainty regarding assessment. We found no other relationships between the target's attractiveness and formidability level in the context of visual attention to whole faces.

Keywords: attractiveness; formidability; face perception; eye-tracking; intrasexual selection; intersexual selection

1. Introduction

People tend to spontaneously assess others for various characteristics, including those relevant to mate choice or conflicts (Little, 2014; Třebický et al., 2019). Based on these assessments, individuals can evaluate the suitability of potential partners or formidability of rivals and take appropriate decisions (Sell et al., 2012; Thornhill & Gangestad, 1999). These assessments are often based on visual cues and face is a particularly salient source of information (Calder et al., 2011).

It has been proposed that inter- and intrasexual selection jointly drive sexual dimorphism of human faces (Puts, 2010; Třebický et al., 2012; Třebický & Havlíček, 2021). Intersexual selection influences facial traits which are considered attractive and believed to provide cues to an individual's mating quality, such as health or an immunocompetence (Stephen & Luoto, 2023 but see Jones et al., 2021; Pátková et al., 2022). Mating with individuals who have attractive traits can have both direct (e.g., parental care, access to resources) and indirect benefits (genetic material for an offspring), and thus increase an individual's reproductive success (Kirkpatrick & Ryan, 1991; Little, 2014). Intrasexual selection, other hand, has probably shaped the development of morphological traits associated with success in confrontations, i.e., traits linked to perceived and actual formidability and dominance (Puts, 2010; Třebický et al., 2012). Formidability assessments are crucial for deciding whom to recruit as an ally and whether to engage in or avoid a physical conflict (Třebický et al., 2021). In other words, they help in deciding whether one stands a good chance of winning (and gaining access to mates and resources) or it is preferable to avoid defeat in a confrontation (and associated possible injuries).

Given the higher prevalence of physical aggression among men, intrasexual selection has been studied more frequently in men (Archer, 2004), but formidability assessments of men are relevant to women as well. For instance, the ability to assess formidable men can help both men and women to stay out of harm's way. Formidability can also serve as a cue of mate quality: a formidable partner may provide advantages, including protection of offspring or heritable traits of formidability (Třebický et al., 2012). This choice, however, also carries potential costs: for instance, research suggests that formidable men need not be better at providing parental care or resources and that their aggression can be directed at their partners (Qvarnström & Forsgren, 1998; Snyder et al., 2008). Both men and women thus have good reasons to pay attention to visual cues of formidability and attractiveness in men. For women, both traits may primarily provide cues to the quality of potential mates, whereas for men, they can help assess the quality of potential rivals.

Multiple studies have investigated the role of individual facial features in attractiveness and formidability judgements. They identified several facial regions – such as the eyes, the nose, mouth, chin, or jaw – whose shape (e.g., wider mouths and fuller lips, more angular jaws, thicker eyebrows) is in men associated with a higher perceived attractiveness (e.g. Windhager et al., 2011) and formidability (e.g., a broader chin, bigger nose and mouth, deep-set eyes, prominent eyebrows) (Třebický et al., 2013). Further, it has been shown that the shape of certain features (e.g., smaller eyes, shorter nose, or wider and more prominent lower jaw) is linked to a higher perceived facial masculinity and dominance (Windhager et al., 2011). Nevertheless, while these morphometrics-based perception studies did find associations between various facial features and judgements of socially relevant characteristics, direct evidence regarding visual attention to these features remains limited.

Research frequently uses eye tracking in order to investigate visual attention directly and to avoid potential biases associated with self-reports. Eye tracking enables the collection of multiple measures of visual attention to areas of interest (AOI) within a visual stimulus, such as the number of fixations, i.e., the number of times the gaze rests at a particular location or AOI, and dwell time in the AOI, which sums the time spent looking at a particular AOI. A higher number of fixations and longer dwell time on a stimulus are interpreted as indicative of its importance, informativeness, likability, and possibly also of the associated cognitive load (Duchowski, 2017; Skaramagkas et al., 2023).

Previous eye-tracking studies have provided some insights into visual attention to others. They showed that when presented with full-body photographs, both men and women direct their visual attention primarily to faces (Hewig et al., 2008). When visually exploring a face without a particular task, the eyes, the nose, and the mouth attract the most visual attention (Hickman et al., 2010; Król & Król, 2019; Semmelmann & Weigelt, 2018). Fewer studies have focused on visual attention to faces and their features within specific contexts. Zhang et al. (2017) reported that during attractiveness ratings of women's faces, men and women looked the most at the nose, eyes, and lips. However, these were the only AOIs specified, which prevented obtaining information about how visual attention towards other features is allocated. Kwart et al. (2012) explored more AIOs and focused on differences in visual attention to faces and their features during ratings of age and attractiveness. They, too, found that in both tasks the majority of visual attention was directed towards the eyes and the nose. Similarly, Hermens et al. (2018) reported that during judgements of trustworthiness and dominance, visual attention is similar in both tasks, with the eyes, the nose, and the mouth attracting the gaze the most. On top of that, it has been demonstrated that more attractive faces and their features (the nose and the mouth, Kwart et al., 2012) are looked at longer and more often, and that this effect is probably stronger in assessments of faces of the preferred sex (Leder et al., 2016; Mitrovic et al., 2018; Valuch et al., 2015).

These findings contrast with the results of morphometric studies, which identified variation in other facial features, especially the jaw and the chin, as affecting both attractiveness and formidability judgements. Still, evidence regarding direct visual attention to particular facial areas in attractiveness and formidability judgements remains equivocal. Eye-tracking studies tend to employ a limited number of AOIs and rarely investigate visual attention during attractiveness rating and even less so during formidability judgements. Moreover, no eye-tracking studies so far investigated the impact of different levels of formidability on visual attention. Exploration of these topics should help us understand the processes underlying visual attention, which may be differentially directed to certain facial features significant in the evolution of human mate choice and competition.

In the present study, we have employed eye tracking to examine men's and women's visual attention (the number of fixations and dwell time) to male faces and particular facial features during assessments of facial attractiveness and formidability. We have also investigated whether and how raters' visual attention differs depending on the level (low, medium, high) of target's facial attractiveness and formidability. To do so, we used life-sized high-resolution facial photographs with multiple AOIs. In addition to the eyes, the nose, and the mouth, we specified also other areas which research had shown to be relevant to facial attractiveness and formidability judgements, such as the cheeks and chin. We predicted that men's and women's visual attention to whole faces would increase (more fixations, longer dwell time) with increasing levels of targets' facial attractiveness and formidability. Further, we predicted that majority of visual attention would be directed to the eyes, the nose, and the mouth, but other facial regions would also attract visual attention. Finally, we have explored possible differences in visual attention to whole faces and particular facial features in relation to the raters' sex and targets' levels of facial attractiveness and formidability.

2. Methods

The study was preregistered prior to data analysis (https://osf.io/5fnc2 and approved by the IRB at the National Institute of Mental Health (ref. num. 111/18). All procedures were conducted in accordance with the Helsinki Declaration. Before enrolling in the study, all participants were informed about its goals and signed an informed consent form. Data used in this study are part of a larger project focused on neural and attentional processes in the visual perception of male faces and bodies using functional magnetic resonance imaging (fMRI) and eye tracking. Data collection took place in Q3–Q4 2019.

2.1. Procedure

The data collection consisted of two consecutive sessions. In the first session, participants had undergone an fMRI scanning session during which they rated the attractiveness and formidability of photographs of 45 male faces and 45 male bodies. This was followed by an eye-tracking session during which they likewise rated photographs of 45 male faces and 45 male bodies on 7-point scales for attractiveness and formidability. In both cases, the stimuli were rated for formidability and attractiveness consecutively, whereby the order of these two large blocks was randomized. Further randomization was then applied within the blocks (for details, see section 2.5. Stimuli blocks). Finally, participants were asked to complete a brief survey regarding their basic demographic data, such as age and sex. After the session, all participants received a debriefing leaflet with a detailed description of the study and 400 CZK (app. 16 EUR) in compensation for their time. The whole session lasted app. 2 hours. Only data from eye tracking of photographs of the faces are considered in this paper.

2.2. Raters

We have recruited participants via social media (Facebook), oral invitations, and posters in the halls of the National Institute of Mental Health, the Faculty of Science, Faculty of Humanities, Faculty of Physical Education and Sports, and student dormitories (all Charles University, Prague, Czechia). Requirements for participation were the following: being a healthy man or woman aged 18–40 years, having normal or corrected-to-normal vision (up to ± 5 diopters), not being pregnant, being right-handed, and without a history of seizures, significant head trauma, mental retardation, claustrophobia, or any other MRI contraindication (such as having metallic or electronic implants; Spaniel et al., 2016).

In total, 40 women and 40 men participated in the study but three of the men were above 40 years of age and therefore removed from further analyses. The resulting sample of raters thus consisted of 40 women (M = 24.9 ys, SD = 5.45, age range = 18–40) and 37 men (M = 24.8, SD = 4.15, age range = 19–38). Age did not statistically discernibly differ between the sexes. For details, see Table S1 in Supplementary Materials.

2.3. Stimuli

As stimuli, we have used 45 standardized facial photographs of men (M = 26.6 ys, SD = 5.86, age range = 18–38) obtained in previous studies (Třebický et al., 2018, 2019). All photographs were post-processed in Adobe Lightroom CS6 and Adobe Photoshop CC 2017 software for standardization of position (while preserving relative differences in head size between individuals), color, and exposure. The facial photographs were then exported in 1:1 scale into 8-bit JPEG format (3840 x 2160, 163 PPI, sRGB color space). For more details, see Třebický et al. (2018).

2.4. Stimuli reference rating

The stimuli were rated for attractiveness and formidability in previous studies on 7-point scales (e.g., 1 – not attractive, 7 – very attractive) (Třebický et al., 2018). Based on this rating, we created categories of faces with low (attractiveness rating: M = 2.17, SD = 0.3; formidability rating: M = 2.8, SD

= 0.27), medium (attractiveness rating: M = 3.02, SD = 0.26; formidability rating: M = 3.8, SD = 0.33), and high (attractiveness rating: M = 4, SD = 0.35; formidability rating: M = 4.9, SD = 0.5) level of attractiveness and formidability. Each category consisted of 15 faces.

2.5. Stimuli blocks

The design of stimuli presentation was analogous in the eye-tracking and the fMRI session to enable possible future comparisons and ensure complementarity of the studies. Stimuli images were presented in blocks. Aside from facial and body photographs, participants were also presented with a set of shuffled images in the form of mosaic pixelated stimuli images created by overlaying individual stimuli images from each stimulation set (5 images per set, 45 sets in total). These were not rated: they functioned as a resting condition (for further details see https://osf.io/u48tz).

). Participants were thus presented with blocks of stimuli which varied in salience (high, medium, low) and with resting non-stimuli (the shuffled images) (Clark et al., 1998). Each rater was presented with 135 images in 27 randomized blocks (9 blocks of faces, 9 blocks of bodies, and 9 blocks of shuffled images) each of which contained 5 images in a randomized order; cf. Fig. 1.

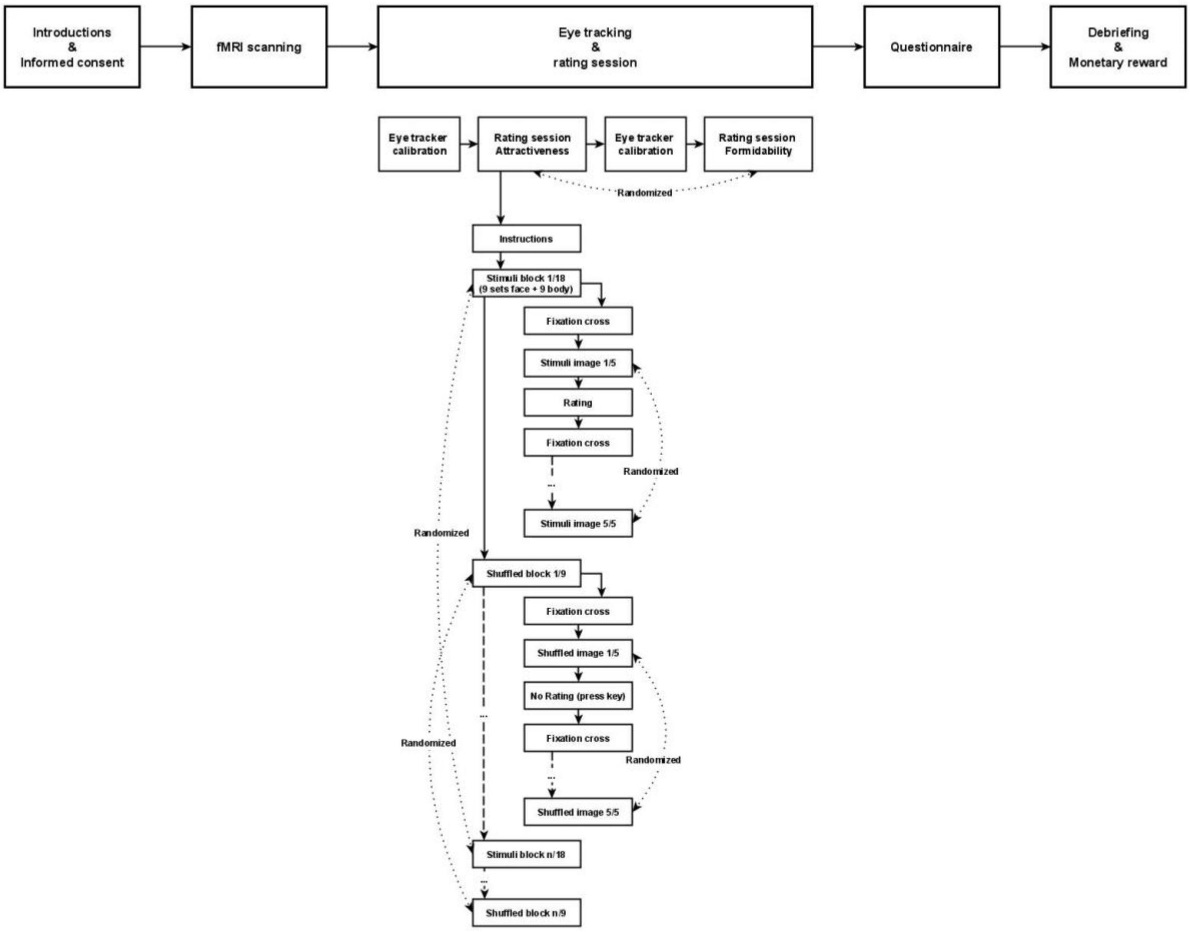

Fig. 1: Procedure flow chart

2.6. Eye tracking

The eye-tracking session took place in a quiet and windowless eye-tracking lab at the National Institute of Mental Health under standardized conditions (with artificial lighting). First, we determined the dominant eye of each participant using a variation of the Porta test (Crovitz & Zener, 1962). During the rating, we then tracked the dominant eye. Raters were seated in an office chair and rested their head on a head-and-chin rest (app. 109cm from the screen, SR Research Head Support) to minimize any movement or change of position. We performed an eye-tracking calibration and validation using a 9-point calibration scheme (Blais et al., 2008). The rating session started only after calibration was successfully validated. successful calibration validation was reached. The 109 cm distance from the screen and 4K resolution results in areas of foveal (center of the field of vision with the highest visual acuity, ~2°angle of view) and parafoveal (area surrounding fovea with lesser vision acuity; ~10 °angle of view) vision (Ivancic Valenko et al., 2020) covering 2% and 52% (40,454px and 1,016,288 px) of the average stimulus face area, respectively (Fig. 2).

The rating task was created and conducted using the Experiment Builder (SR Research) on a 4K 27" LCD screen (Benq IPS; 3840x2160, 163 PPI, 99% sRGB color space coverage) pivoted into portrait orientation to accommodate life-sized facial photographs. The LCD screen was color- and luminance-calibrated using an X-rite i1 Display probe (connected during the experiment). Eye movements were recorded by an EyeLink 1000 Plus eye-tracker (SR Research Ltd. Ottawa, Ontario, Canada) (1000 Hz) and data collected by a host PC running Romdos 7.1 OS.

During the eye-tracking session, participants were instructed to rate the facial photographs using the 7-point scales twice: once for attractiveness ("How attractive do you find the man in the photograph?"; 1- least attractive, 7 – most attractive) and once for formidability ("How successful would be this man in a physical encounter confrontation"?; 1 – least successful, 7 – most successful). The order of the tasks (attractiveness/formidability rating) was randomized.

Participants were first instructed to look at a fixation cross displayed for 1,000ms in different quadrants of the screen (but not in the center of the screen to avoid fixations bias for the area where the stimuli were about to be presented). This was followed by a 4,000ms-long presentation of facial photograph. On the following screen, participants were shown a 7-point verbally anchored scale of attractiveness/formidability, which they used to indicate their rating by clicking a mouse.

2.7. Delineation of AOIs

We have manually defined an array of AOIs for each stimulus using the Data Viewer software (4.3.1). In addition to the typically used eyes, nose, and mouth, we have also included features identified as potentially important for judgements of attractiveness and formidability by previous studies

(Cunningham et al., 1990; Třebický et al., 2013; Windhager et al., 2011). The final set of AOIs thus included the whole face, the right eye, the left eye, nose, mouth, forehead (including hair), chin, the right cheek, the left cheek, the right ear, and the left ear. This set is similar to one used by Chelnokova & Laeng (2011). See Fig. 2 for individual AOIs; for AOI areas in px, see Table S2 in Supplementary Materials.

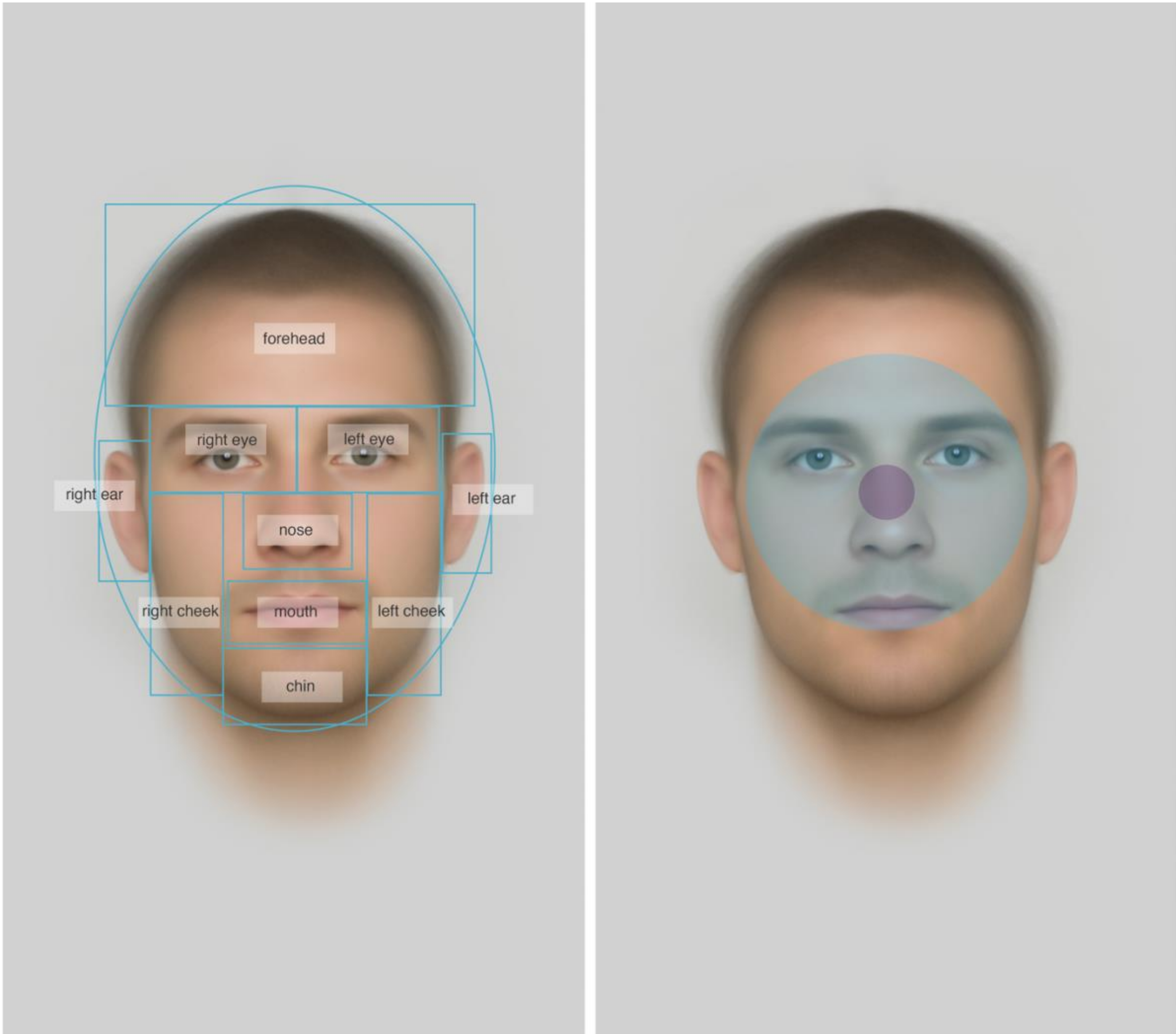

Fig. 2: An example of delineated AOIs on the left (the ellipse indicates the "whole face" AOI) and visualization of the central vision on the right. The violet color represents an estimate of foveal vision, the blue color parafoveal vision, which jointly amount to central vision.

3. Data analyses

All statistical analyses were performed in jamovi (v 2.3.28) (The Jamovi Project, 2023). As in previous studies (e.g., Millen & Hancock, 2019), we considered only fixations ≥80 ms in the analyses. Aside from that, we noticed that for three raters, no fixations were recorded at some point during attractiveness rating. For the first rater, this occurred for five targets; for the second rater, it was the case for 20 targets. For the third rater, it occurred for one target. In the formidability rating, this happened for a combination of one rater and one target. All these cases were likely the result of eye-tracking signal loss, which is why we have excluded these trials from subsequent analyses (Leder et al., 2016; Rudolfová et al., 2022).

Visual inspection of the data distribution and the Kolmogorov–Smirnov test indicated that the data for the number of fixations and dwell time did not follow a normal distribution but a negative binomial. Therefore, we employed generalized mixed-effects models (GLMMs) using the GAMLj module (v 2.6.6) in jamovi.

The contexts (attractiveness/formidability ratings) were analyzed in separate models; visual attention to the whole face and to the individual AOIs was likewise analyzed separately. In separate models, the number of fixations and dwell time (ms) were entered as dependent variables.

The target's level of attractiveness/formidability (high, medium, low) and the rater's sex were entered as predictors for whole-face analyses. For example, the relationship between the rater's number of fixations on the whole face (a dependent variable), rater's sex, target's level of facial attractiveness (both predictors), and their interaction during facial attractiveness rating were assessed using the following model: N fixations on face ~ 1 + target's attractiveness level + rater's sex + target's attractiveness level:rater's sex + (1|ID rater). For formidability ratings, we used an analogous model. Subsequently, we ran models that included dwell time on whole faces. To control for the variability of targets and raters, we have first entered the rater's and the target's ID as random effects. Those models, however, exhibited a singular fit, which was due to virtually no variance of ID target and generally had a lower Akaike information criterion (AIC) when only the rater ID random effect term was included. Therefore, we report all analyses without the target ID random effect. For details, see https://osf.io/5fnc2.

For analyses of individual AOIs, we have entered as predictors the target's levels of attractiveness/formidability, the rater's sex, and the AOI. Based on the AIC or because the models did not converge when the ID target was included, we include only the ID rater as a random effect in the model. For details, see https://osf.io/5fnc2.

In a minor divergence from preregistration, we report X^2 and p-value for fixed-effect omnibus test results in the main text for the GLMMs, while fixed-effect parameter estimates using (exp)B with 95% CI for each model can be found in the Supplementary Materials. We used a post hoc test with Holm correction to test differences between pairs of predictor levels. Full results and observed power (Table S3) are reported in the Supplementary Materials.

We have set $p \leq 0.05$ as a threshold for statistical discernibility. By using the term "statistically discernible" instead of traditionally used "statistical significance", we want to indicate that the statistical test has found some evidence of a discernible effect but avoid any implication of its significance.

4. Results

4.1. Whole face analyses

4.1.1. Attractiveness

A GLMM showed that during the facial attractiveness rating task, the *number of fixations* on the whole face was not statistically discernibly predicted by the target's level of facial attractiveness (X^2 (2) = 0.005, p = 0.997), the rater's sex (X^2 (1) = 0.360, p = 0.549), or by an interaction of the two (X^2 (2) = 0.371, p = 0.831) (Fig. 3).

A further GLMM showed that during the facial attractiveness rating task, the *dwell time* on the whole face was not statistically discernibly predicted by the target's level of facial attractiveness (X^2 (2) = 3, p = 0.223). It was, however, predicted by the rater's sex (X^2 (1) = 8.08, p = 0.004): men spent statistically discernibly more time looking at whole faces than women did (3,308ms vs 3,177ms) when assessing male facial attractiveness (Table 1 and Fig. 3).

4.1.2. Formidability

A GLMM showed that during the facial formidability rating task, the *number of fixations* on the whole face was not statistically discernibly predicted by the target's level of facial formidability (X^2 (2) = 1.128, p = 0.569) or rater's sex (X^2 (1) = 0.002, p = 0.962) (Fig. 2). For the *dwell time* during facial formidability rating of the whole face, the model showed that it was statistically discernibly predicted by the target's formidability level (X^2 (2) = 9.84, p = 0.007) and by rater's sex (X^2 (1) = 6.99, p = 0.008) (Fig. 2), but there was no statistically discernible interaction between the two (X^2 (2) = 1.67, p = 0.435). During facial formidability assessment, both men and women exhibited statistically discernibly but just marginally longer dwell time on men's faces with a medium rather than high level of facial formidability (3,233ms vs 3,193ms). Men also had slightly longer dwell time than women (3,281ms vs 3,153ms) during this task; see Table 1 and Fig. 3.

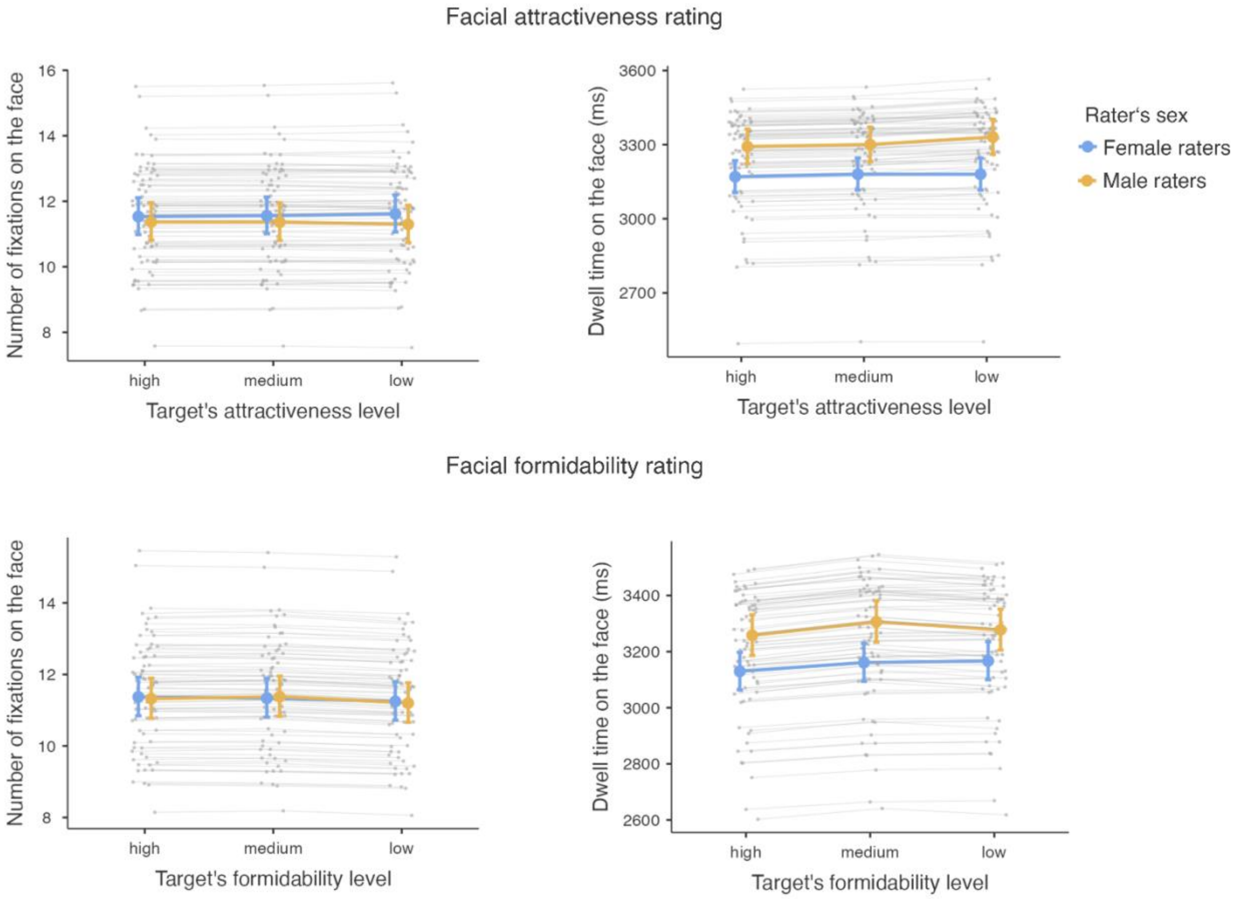

Fig. 3: Differences in visual attention in relation to the target's facial attractiveness level during facial attractiveness rating (top row) and in relation to the target's facial formidability level during facial formidability assessment (bottom row), with the number of fixations on the whole face on the left and dwell time on the whole face on the right side. Colored dots represent mean values, error bars their 95% confidence intervals, blue color represents female raters, and yellow color male raters. Grey lines and dots represent random effects plotted by the ID rater.

4.2. AOI analyses

4.2.1. Attractiveness

A GLMM showed that the *number of fixations* in AOIs during the facial attractiveness rating task was statistically discernibly predicted by the AOI (X^2 (9) = 20,483.46, p < 0.001) and by the target's level of attractiveness (X^2 (2) = 9.87, p = 0.007), but not by rater's sex (X^2 (1) = 3.74, p = 0.053). Interaction between AOIs and rater's sex (X^2 (9) = 140.83, p < 0.001) as well as interactions between AOIs and the target's level of attractiveness (X^2 (18) = 108.21, p < 0.001) were also statistically discernible. Interaction between rater's sex and target's level of attractiveness (X^2 (2) = 1.70, p = 0.428) and between AOI, rater's sex, and target's level of attractiveness (X^2 (18) = 17.08, p = 0.518) were, however, not statistically discernible. Men had on average statistically discernibly more fixations than women on the chin (0.144 vs 0.089), forehead (1.138 vs 0.839), and the left ear (0.123 vs 0.083). Regardless of their sex, participants made on average statistically discernibly more fixations on the chin of faces with high rather than low attractiveness level (0.149 vs 0.093) and on the left cheek of faces with a medium rather than low attractiveness level (0.107 vs 0.061). Further, regardless of sex, participants had on average statistically discernibly more fixations on the left ear of highly attractive as opposed to medium attractive faces (0.145 vs 0.071) and more fixations on the right ear of faces with a low rather than medium level of attractiveness (0.078 vs 0.035). For details, see Table 1 and Fig. 4.

In terms of the *dwell time* in AOIs during facial attractiveness rating, GLMM had shown that it is statistically discernibly predicted by the AOI (X^2 (9) = 9,535.77, p < 0.001) and by target's level of attractiveness (X^2 (2) = 6.81, p = 0.033) but not by the rater's sex (X^2 (1) = 3.23, p = 0.072). There was also a statistically discernible interaction between the AOI and rater's sex (X^2 (9) = 58.46 p < 0.001) and between the AOI and target's level of attractiveness (X^2 (18) = 142.43, p < 0.001), but not between rater's sex and target's level of attractiveness (X^2 (2) = 2.12, p = 0.346) or between the AOI, rater's sex, and target's level of attractiveness (X^2 (18) = 23.84, p = 0.160). Men exhibited on average statistically discernibly longer dwell time on the chin and the left ear (37.8ms and 30.8ms, respectively) than women did (20.4ms and 18.5ms, respectively). Further, regardless of sex, participants exhibited a statistically discernibly longer dwell time on the chin of faces with a high rather than low attractiveness level (39.74ms vs 21.04ms) and on the left cheek of faces with a medium rather than high or low level of attractiveness (26.07ms, 14.19ms, 13.31ms, respectively). Moreover, raters exhibited a longer dwell time on the left ear of highly attractive rather than medium attractive faces (35.90ms vs 14.86ms) and on the left ear of faces with a low as opposed to medium level of attractiveness (25.47ms vs 14.86ms). Finally, raters had a longer dwell time on the right ear of faces with a low as opposed to medium level of attractiveness (15.92ms vs 6.39ms) and on the right ear of faces with a high rather than medium level of attractiveness (11.11ms vs 6.39ms). For details, see Table 1 and Fig. 4.

4.2.2. Formidability

A GLMM showed that the *number of fixations* in AOIs during facial formidability rating was statistically discernibly predicted by the AOI (X^2 (9) = 21,353.83, p < 0.001), target's formidability level (X^2 (2) = 21.18, p < 0.001), rater's sex (X^2 (1) = 11.58, p < 0.001), by interaction between the AOI and target's level of formidability (X^2 (18) = 109.03, p < 0.001), and by interaction between the AOI and rater's sex (X^2 (9) = 118.09, p < 0.001). It was not, however, predicted by interaction between the target's level of formidability and rater's sex (X^2 (2) = 3.84, p = 0.147) or by interaction between the AOI, target's level of formidability, and rater's sex (X^2 (18) = 27.52, p = 0.070). Men made on average statistically discernibly more fixations on the chin than women (0.186 vs 0.118 for men and women respectively), more fixations on the forehead (1.002 vs 0.828), more fixations on the left cheek (0.116 vs 0.062), and more fixations on the right cheek (0.120 vs 0.083). Regardless of sex, participants made statistically discernibly more fixations on the chin of faces with a high as opposed to low level of formidability (0.194 vs 0.120) and on the left cheek of faces with a medium as opposed to low level of formidability (0.112 vs 0.058). Moreover, they made more fixation on the left ear of faces with a high as opposed to medium level of formidability (0.155 vs 0.088) and more fixations on the right ear of faces with a low as opposed to medium level of formidability (0.119 vs 0.060). For details, see Table 1 and Fig. 4.

The GLMM showed that the *dwell time* on AOIs during facial formidability rating was statistically discernibly predicted by the AOI (X^2 (9) = 7,592.15, $p < 0.001$), the target's level of formidability (X^2 (2) = 15.14, $p < 0.001$), rater's sex (X^2 (1) = 4.45 $p = 0.035$), by interaction between the AOI and target's level of formidability (X^2 (18) = 156.64, $p < 0.001$), and by interaction between the AOI and the rater's sex (X^2 (9) = 71.29, $p < 0.001$). It was not, however, predicted by interaction between the target's level of formidability and rater's sex (X^2 (2) = 2.07, $p = 0.355$) or by interaction between the AOI, target's level of formidability, and rater's sex (X^2 (18) = 8.19, $p = 0.976$). Men exhibited an on average statistically discernibly longer dwell time on the chin (45.87ms vs 27.26 for men and women respectively), the left cheek (26.17ms vs 14.14ms), and the right cheek (26.24ms vs 13.72ms). Further, regardless of sex, participants had a statistically discernibly longer dwell time on the chin of faces with a high as opposed to low level of formidability (48.70ms vs 27.81ms), on the left cheek of faces with a medium as opposed to low level of formidability (29.12ms vs 12.56ms), on the left ear of faces with a high as opposed to medium level of formidability (35.99ms vs 15.71ms), on the right ear of faces with a high as opposed to medium level of formidability (14.42 vs 6.67ms), and on the right ear of faces with a low as opposed to medium level of formidability (21.09 vs 6.67ms). For details see Table 1 and Fig. 4.

Measure	AOI	Attractiveness		Formidability	
		Female raters	Male raters	Female raters	Male raters
		Mean with 95% CI [LL, UL]		Mean with 95% CI [LL, UL]	
Number of fixations	Whole face	11.6 [11.1, 12.1]	11.3 [10.8, 11.9]	11.3 [10.8, 11.8]	11.3 [10.8, 11.8]
	Right eye	3.32 [3.14, 3.50]	3.01 [2.84, 3.18]	3.26 [3.09, 3.43]	3.07 [2.91, 3.25]
	Left eye	3.43 [3.25, 3.62]	3.33 [3.15, 3.52]	3.29 [3.12, 3.47]	3.39 [3.21, 3.58]
	Nose	1.86 [1.76, 1.98]	1.82 [1.71, 1.93]	1.90 [1.80, 2.01]	1.73 [1.63, 1.83]
	Mouth	1.12 [1.05, 1.19]	1.01 [0.94, 1.08]	0.97 [0.91, 1.03]	0.88 [0.82, 0.94]
	Forehead	0.84 [0.78, 0.90]	1.14 [1.06, 1.22]	0.83 [0.77, 0.89]	1.00 [0.94, 1.07]
	Chin	0.09 [0.08, 0.11]	0.14 [0.13, 0.17]	0.12 [0.10, 0.14]	0.19 [0.16, 0.21]
	Right cheek	0.09 [0.07, 0.10]	0.07 [0.06, 0.08]	0.08 [0.07, 0.10]	0.12 [0.10, 0.14]
	Left cheek	0.07 [0.06, 0.08]	0.09 [0.07, 0.10]	0.06 [0.05, 0.08]	0.12 [0.10, 0.14]
	Right ear	0.06 [0.05, 0.07]	0.05 [0.04, 0.07]	0.10 [0.08, 0.12]	0.08 [0.07, 0.10]
	Left ear	0.08 [0.07, 0.10]	0.12 [0.11, 0.14]	0.11 [0.10, 0.13]	0.13 [0.11, 0.15]
Dwell time (ms)	Whole face	3177 [3116, 3239]	3308 [3242, 3375]	3153 [3089, 3218]	3281 [3212, 3351]
	Right eye	1030.5 [848.47, 1251.5]	957.9 [783.72, 1170.7]	1065.81 [853.84, 1330.4]	1023 [812.87, 1287.4]
	Left eye	1150.7 [944.86, 1401.3]	1129.1 [920.45, 1385.1]	1241.59 [993.06, 1552.3]	1177.11 [934.99, 1481.9]
	Nose	486.8 [399.42, 593.2]	498.1 [405.7, 611.5]	571.89 [457.55, 714.8]	471.04 [374.22, 592.9]
	Mouth	325.5 [267.24, 396.6]	280.6 [228.87, 344]	307.55 [246.12, 384.3]	261.86 [208.04, 329.6]
	Forehead	208.5 [171.2, 254]	325.4 [265.27, 399.3]	212.92 [170.68, 265.6]	286.1 [227.33, 360.1]
	Chin	20.4 [16.72, 25]	37.8 [30.78, 46.5]	27.26 [21.77, 34.1]	45.87 [36.39, 57.8]
	Right cheek	14.7 [11.92, 18]	15.8 [12.86, 19.5]	13.72 [10.96, 17.2]	26.24 [20.79, 33.1]
	Left cheek	13.9 [11.32, 17]	20.8 [16.98, 25.6]	14.14 [11.3, 17.7]	26.17 [20.76, 33]
	Right ear	10.5 [8.54, 12.9]	10.3 [8.39, 12.8]	9.98 [7.89, 12.6]	16.05 [12.7, 20.3]
	Left ear	18.5 [15.11, 22.7]	30.8 [25.03, 37.8]	18.76 [14.92, 23.6]	30.41 [24.12, 38.3]

Table 1: The number of fixations and dwell time in AOIs for each context (attractiveness and formidability) and sex. Mean values are calculated based on the estimated marginal means of each respective model.

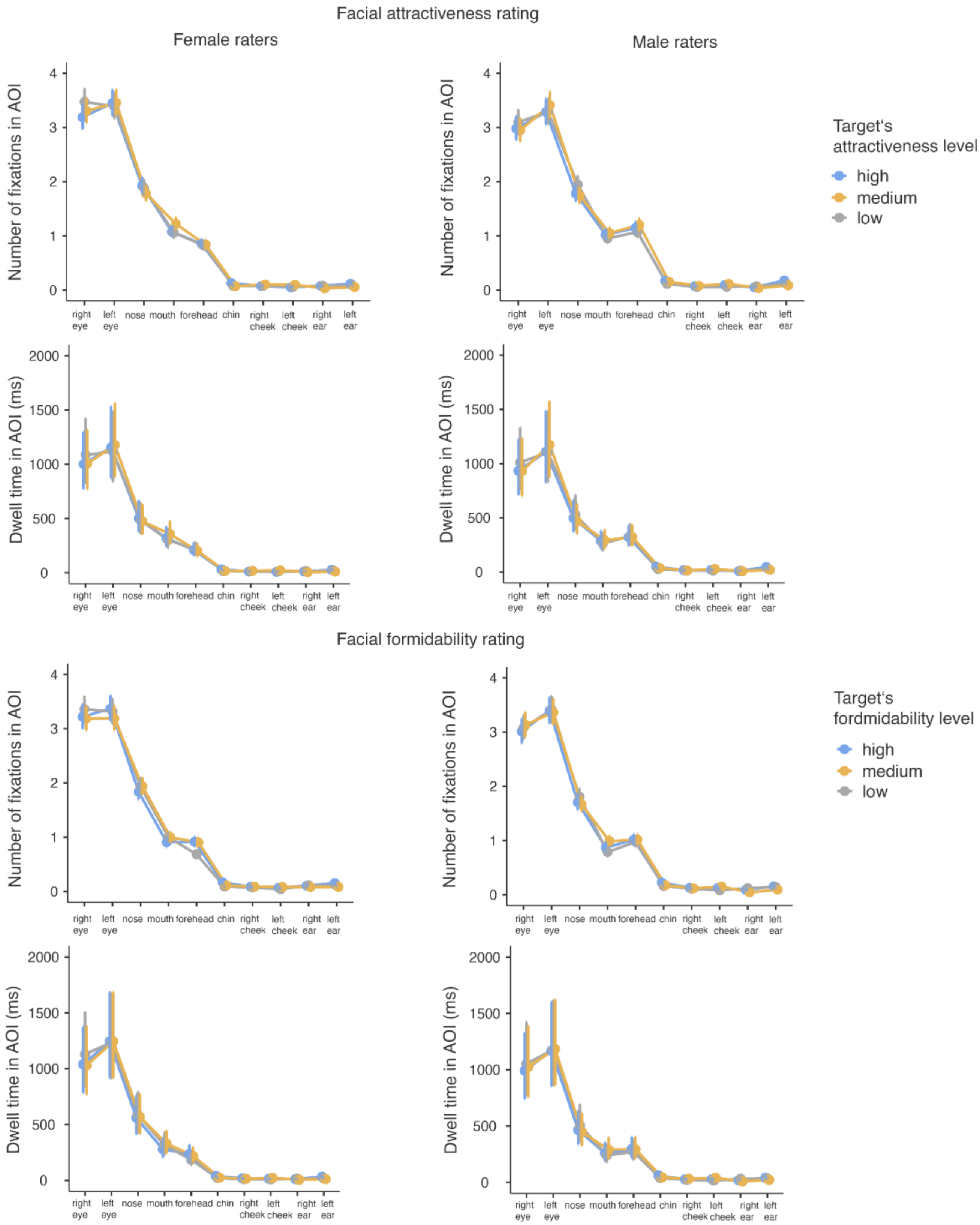

Fig. 4: Differences in visual attention directed at individual AOIs in relation to the target's level of attractiveness during a rating of facial attractiveness (top) and facial formidability (bottom). Female

raters are in the left column, male raters in the right one. For each rating, the number of fixations on AOIs is in the top row and the dwell time in AOIs in the bottom row. Dots represent mean values, error bars show their 95% confidence intervals.

5. Discussion

The main aim of the present study was to investigate men's and women's visual attention (measured as the number of fixations and dwell time) directed at real-life-sized photographs of male faces during attractiveness and formidability rating. We have also explored whether and how the target's level of attractiveness and formidability (low, medium, high) affects raters' visual attention during each of the two kinds of judgements. In addition to the eyes, the nose, and the mouth, i.e., areas typically used in eye-tracking studies, we have defined a broad array of AOIs proposed by various morphometric studies. We found that during both types of judgements, men looked longer at the faces than women did. Raters of both sexes also looked longer at faces with a medium rather than high formidability level. Irrespective of the characteristics rated, both sexes paid the most visual attention to the eyes, nose, mouth, and forehead. Aside from that, we found statistically discernible variations also for other facial regions, such as the chin, the cheeks, and the ears.

5.1. Whole face analyses

Contrary to our predictions, analyses of visual attention to the whole face showed that during attractiveness ratings, there was no association between the number of fixations on the face, target's level of attractiveness, rater's sex, or their interaction. Nevertheless, men showed a statistically discernibly longer dwell time during attractiveness rating than women did. For the formidability rating, again contrary to our expectations, we found no statistically discernible relationships for the number of fixations on whole faces. On the other hand, the dwell time on the whole face during formidability rating was predicted by the rater's sex (men had marginally longer dwell times on faces than women did) and by the target's formidability level (raters looked longer on faces with a medium rather than high level of facial formidability). These patterns do not follow our prediction according to which visual attention would increase with the target's higher level of attractiveness or formidability.

The absence of a relationship between the number of fixations on the whole face, target's attractiveness level, and rater's sex is somewhat surprising because previous research tended to report that attractive faces are looked at more often and longer (Leder et al., 2016; Mitrovic et al., 2018). Even so, some studies (Leder et al., 2016; Mitrovic et al., 2018) reported merely a rather weak association between visual attention and attractiveness of male faces and in the study by Mitrovic et al. (2016), it was not observed at all. Our results thus do have a precedent.

Why women's visual attention to male faces did not differ in relation to the level of perceived attractiveness is more puzzling. Possible explanation might be that previous studies which demonstrated this association (e.g., Leder et al., 2016; Mitrovic et al., 2016, 2018) used different designs than our study. For instance, they used photographs with two people in one picture, with natural-looking settings and used a free-viewing paradigm – such setups may have facilitated a comparison between the stimuli faces and allow for the more attractive stimulus to capture rater's visual attention more. Moreover, it is also possible that in our stimuli, the differences in attractiveness were not pronounced enough to generate a significant effect on visual attention, although differences between the mean ratings in individual categories do seem similar to previous studies (at least with respect to the high and low categories) which were able to demonstrate the effect (Kwart et al., 2012; Mitrovic et al., 2016).

Interestingly, we found that men had a marginally longer dwell time (Δ ~130ms) on male faces during facial attractiveness ratings than women did. This may indicate the task difficulty. Men may need more time to extract the relevant information in such contexts (Jacob & Karn, 2003), especially if they are not used to judging other men's attractiveness. Moreover, Mitrovic et al. (2016) suggested intrasexual comparison as an explanation of why men spend more time looking at male faces regardless of differences in facial attractiveness levels. This might also apply to our case: men may have been comparing themselves to the stimuli, thus needing more time for the assessment, while women did not. Along similar lines, that is, based on intrasexual comparison, we might explain the marginally longer dwell time of male participants during formidability judgements (Δ ~128ms). In other words, we can speculate that in our male raters, the assessment of both male facial attractiveness and formidability may have been associated with a comparison between themselves and the target, and this translated to a longer gaze. In future studies, the use of both male and female stimuli may help resolve this issue. Further, raters looked longer at faces with a medium (as opposed to high) level of formidability, which may be the result of the ambiguity of these faces and raters' uncertainty regarding their rating. This may have led to the prolonged gaze duration (Martín-Loeches et al., 2014). In any case, differences in dwell duration were rather small (Δ ~40ms) and one could argue about their practical significance.

5.2. Analyses of areas of interest

Analyses which explored visual attention directed at individual AOIs showed that for attractiveness rating, the highest number of fixations and the longest dwell time were, as expected, directed at the eyes, nose, mouth, and also forehead (in our case including the top of a head and hair) (Table 1 and Fig. 4). The remaining areas received comparatively little direct visual attention. An exploration of interactions showed that men made marginally more fixations on the chin, forehead, and the left ear and had a longer dwell time on the chin and left ear than women did. Further, raters, irrespective of sex, made marginally more fixations and had a longer dwell time on the chin of faces with a high as opposed to low level of attractiveness and on the left cheek of faces with a medium as opposed to low level of attractiveness. Longer dwell times have also been found for the left cheek of faces with a medium as opposed to high attractiveness levels. Finally, we have observed some variation in the number of fixations and dwell time on the left and right ear in relation to the target's level of attractiveness. But given that these differences were only in the order of fractions of fixations and tens of milliseconds, it would be farfetched to argue about their practical implications.

Analyses that investigated visual attention directed at particular AOIs showed that for formidability rating, the highest number of fixations and the longest dwell time were, as expected, likewise directed at the eyes, the nose, the mouth, and the forehead. Exploration of interactions had shown that men had statistically discernibly more fixations and longer dwell time on the chin and both cheeks and more fixations on the forehead than women did. Raters made marginally more fixations and had a longer dwell time on the chin of faces with a high as opposed to low level of formidability and on the left cheek of faces with a medium as opposed to a low level of formidability. In parallel to the attractiveness ratings, we found a variation for the number of fixations and dwell time on the right and the left ear in relation to the target's level of formidability. As in the case of interactions within attractiveness ratings, the observed differences were small and one might once again dispute their practical relevance.

Overall, the observed patterns of visual attention align with previous eye-tracking studies: they show that the eyes, the nose, and the mouth regions receive the most visual attention regardless of the context (i.e., characteristics being rated) (Chelnokova & Laeng, 2011; Hermens et al., 2018; Kwart et al., 2012). Some studies have also reported the forehead as an area of increased visual interest (Nguyen et al., 2009). We have likewise observed heightened visual attention towards the forehead in our study, especially in male raters. Windhager et al., (2011) showed forehead shape changes in relation to an individual's strength, but otherwise, the forehead doesn't seem to be commonly connected with perceived formidability. It should be noted that while in our study, the AOI was classified as the forehead, it included hair, which may have attracted raters' visual attention on its own. Our finding that during both attractiveness and formidability judgements, it is mainly the eyes, the nose, and the mouth that attract visual attention contrasts with some perception studies and with the findings of morphometric studies, which showed that the chin and the cheeks may also be important for judgements of these traits (Cunningham et al., 1990; Třebický et al., 2013; Windhager et al., 2011).

There are several possible reasons why the chin and the cheeks attracted only limited visual attention. Morphometric studies show that while the shape of certain (and multiple) facial regions may be associated with the rating of particular characteristics, their perceptual importance can vary. Morphological growths of facial regions are interconnected (Enlow & Hans, 1996), so the shape of one area affects the shape of surrounding regions. For instance, the shape of the masseter muscle influences the shape of the mandible and zygomatic arch and, thus, the shape of cheeks. Morphometric studies may thus detect shape deformation in multiple regions but only some of them affect perception, while others are merely shape correlates. Another reason may have to do with certain limitations of eye-tracking in terms of indicating what raters actually see. Although we used life-sized stimuli (to allow for a more realistic visual search, as opposed to most previous eye-tracking research), which is a strong point of our study, the area covered by central vision may have covered multiple AOIs at once (see Figure 2). As a result, although direct visual attention was not directed specifically at some facial features, they may have been in the rater's field of central vision. It means that raters may have seen them and processed them without the eye-tracker detecting a direct fixation. This is well possible: it has been demonstrated that attractive faces which are even outside a person's foveal vision capture visual attention (Sui & Liu, 2009) and another study has shown that when it comes to identification of attractive faces, there is no difference in the performance between the foveal and parafoveal vision (Guo et al., 2011). On top of that, people can detect attractive faces even using their peripheral vision (Guo et al., 2011).

Nevertheless, men in our sample had slightly more fixations and longer dwell time on the chin, more fixations on the forehead during both formidability and attractiveness ratings, and more fixations on cheeks during formidability judgements than women did. We suggest that these areas might contain information which helps men make the relevant judgements and that these regions are more important to men than they are to women. Finally, some of the variation in visual attention to the ears might be explained by certain specific characteristics of some of our targets. Being MMA fighters, some targets had "cauliflower" ears, which may have attracted some attention, as it might be unexpected to see but also possibly cue formidability. Still, it should be stressed that although we offer interpretations for the observed differences, they are quite small and one ought to be cautious regarding their importance.

Some previous studies have shown that the noses and mouths of attractive faces are fixated upon more often (Kwart, 2012). Our present findings do not bear this out. Instead, we found that the chin of highly formidable and highly attractive faces received more fixations and was looked at longer than these areas in faces with low attractiveness/formidability, and this was also the case for the left cheek of faces with the medium rather than high level of attractiveness/formidability. This might indicate a level of salience of these regions for faces with certain levels of formidability/attractiveness and the relevant judgements. It is also possible that irrespective of the type of judgement, one inspects the eyes, the nose, and the mouth, and in case of uncertainty about the judgement, one inspects further facial features, such as the cheeks and the chin, which then contribute to reaching a decision. Moreover, given that the patterns of visual attention patterns were similar for judgements of both attractiveness and formidability and their respective levels, one could speculate that these two specific judgements involve similar visual attention.

6. Limitations

Our study had certain limitations. We focused on young adults, persons at the stage of life when majority of mate choice takes place. Future studies should also investigate adolescents and seniors to gain insight into the developmental trajectories of visual attention to faces. Another possible limitation might be that our targets were MMA fighters. As such, they constituted a specific sample whose characteristics (e.g., broken noses, cauliflower ears) differed from the general population. The sample may have also suffered from a skewed attractiveness and formidability distribution or insufficient variation in their levels: the interindividual differences may have been too small to translate into measurable differences in visual attention. Moreover, our stimuli presentation design was not fully randomized in the traditional way. The presentation of stimuli in blocks (due to the study being part of a larger project) may have played a role. For instance, a stimuli block contained pictures of the same salience, which could have led to raters' desensitization to the level of stimuli's attractiveness/formidability.

7. Conclusions

We have assessed visual attention to male faces and their particular features in the context of attractiveness and formidability judgements made by both men and women, whereby visual attention was measured both as the number of fixations and the dwell time. We observed that during both facial attractiveness and formidability judgements, men looked longer at faces than women did. Further, we found no effect of raters' attractiveness and formidability level on their visual attention except that raters, irrespective of their sex, looked longer at faces with a medium as opposed to high level of formidability. Moreover, regardless of the rated characteristics, men and women directed the most visual attention to the eyes, nose, mouth, and forehead of the stimuli. Other facial regions received relatively little visual attention. We have detected small variations in visual attention directed at, e.g., the forehead, chin, cheeks, and the ears in relation to the rater's sex and target's level of attractiveness, and while we propose some interpretations, we are aware that these differences, while statistically discernible, need not be large enough to have any practical importance. To conclude, the eyes, the nose, and the mouth seem to be central to the evolution of facial perception as the most salient regions for gathering information about others. Future research should strive to further connect eye tracking and GMM methods and investigate whether areas of important morphological deformations correspond to areas of increased visual attention.

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10. Data availability

The data and Supplementary Materials associated with this research are available at https://osf.io/q6jt7/?view_only=41ce8b59f98743c1ab0a9aca176be925.

11. Author contributions

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CHAPTER 10

CROSS-MODAL ASSOCIATIONS OF HUMAN BODY ODOUR ATTRACTIVENESS WITH FACIAL AND VOCAL ATTRACTIVENESS PROVIDE LITTLE SUPPORT FOR THE BACKUP SIGNALS HYPOTHESIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Cross-modal associations of human body odour attractiveness with facial and vocal attractiveness provide little support for the backup signals hypothesis: A systematic review and meta-analysis

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ABSTRACT

Assessing the attractiveness of potential mating partners typically involves multiple sensory modalities, including the integration of olfactory, visual, and auditory cues. However, predictions diverge on how the individual modalities should relate to each other. According to the *backup signals* hypothesis, multimodal cues provide redundant information, whereas the *multiple messages* hypothesis suggests that different modalities provide independent and distinct information about an individual's mating-related quality. The *backup signals* hypothesis predicts a positive association between assessments based on different modalities, whereas no substantial correlation across modalities is expected under the *multiple messages*hypothesis. Previous studies testing the two hypotheses have provided mixed results, and a systematic evaluation is currently missing.

We performed a systematic review and a meta-analysis of published and unpublished studies to examine the congruence in assessments between human body odour and facial attractiveness, and between body odour and vocal attractiveness. We found positive but weak associations between ratings of body odours and faces ($r = 0.1$, $k = 25$), and between body odours and voices ($r = 0.1$, $k = 9$). No sex differences were observed in the magnitude of effects.

Compared to judgments of facial and vocal attractiveness, our results suggest that assessment of body odour provides independent and non-redundant information about human mating-related quality. Our findings thus provide little support for the *backup signals* hypothesis and may be better explained by the *multiple messages* hypothesis.

1. Introduction

Across many different taxa, individuals assess potential mating partners via telereceptive senses such as vision, olfaction, and hearing (Aglioti & Pazzaglia, 2011). Although some vertebrates appear to rely predominantly on a single sense (Arakawa, Blanchard, Arakawa, Dunlap, & Blanchard, 2008; Candolin, 2003; Gosling & Roberts, 2001), most species, including humans, employ multiple senses (Candolin, 2003;

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Higham & Hebets, 2013) in their assessment. Frog calls, for example, are often accompanied by conspicuous vocal sac movements and/or water surface vibrations, while many bird species show complex, rhythmic and vigorous visual displays during courtship singing (for a review, see Halfwerk et al., 2019).

Perceived variation in these physical traits may provide information about an individual's mating-related quality, such as health and fertility (e.g., Grammer, Fink, Møller, & Thornhill, 2003; Rhodes, 2006; Thornhill & Gangestad, 1999b). As the judgment of an individual's attractiveness based on any single modality entails a certain level of error, using multiple sensory channels could enable a more reliable assessment (Møller & Pomiankowski, 1993). Two competing hypotheses have been proposed to explain the use of multiple modalities in the assessment of potential mates (Groyecka et al., 2017; Higham & Hebets, 2013). According to the '*backup signals*' hypothesis (Grammer, Fink, Juette, Ronzal, & Thornhill, 2001; also coined redundant signalling, Møller & Pomiankowski, 1993; Thornhill & Grammer, 1999), certain cues may provide similar (redundant) information; assessing this same information in several different modalities will then tend to reduce error and facilitate a more accurate overall assessment of underlying quality. In contrast, the *multiple messages* hypothesis (Cunningham, Barbee, & Pike, 1990; Møller & Pomiankowski, 1993) suggests that each trait provides distinct and independent (non-redundant) information about an individual's mating-related quality, but in combination, these can facilitate more accurate assessment of overall individual quality than any single cue in isolation. With all this in mind, we can make predictions to test these two ideas. One can expect that if attractiveness assessments based on different sensory channels are closely and positively associated, such congruence would suggest redundancy in information across traits and provide support for the backup hypothesis. Weak or absent cross-modal congruence (i.e. cues convey non-redundant information), however, would support the multiple messages hypothesis. The mating-related animal research provided some support for both of these hypotheses. The use of backup signals of quality was demonstrated, for instance, in *Drosophila saltans* where removing one courtship component (either visual, auditory, chemical or tactile) did not eliminate the female's decision to mate (Colyott, Odu, & Gleason, 2016). On the other hand, the study on peacock spiders (*Maratus volans*) showed that both visual and vibratory signalling is important for mating success supporting the multiple messages hypothesis (Girard, Elias, & Kasumovic, 2015). Overall, the majority of available animal research seems to provide more evidence in favour of the multiple messages hypothesis (Candolin, 2003).

Most research on human mate preferences has focused on visual cues, typically by investigating people's assessments of facial and/or body attractiveness. Although physical appearance certainly plays a prominent role (Groyecka et al., 2017; Herz & Inzlicht, 2002; Walter et al., 2020), the assessment of attractiveness in potential mating partners is undeniably multimodal. Research suggests that body odour (Havlíček et al., 2008; Roberts et al., 2011) and vocal cues (Hill & Puts, 2016; Pisanski, Feinberg, Oleszkiewicz, & Sorokowska, 2017; Zäske, Skuk, & Schweinberger, 2020) also contribute substantially to human mate preferences (Groyecka et al., 2017). However, studies that examine potential cross-modal congruency and redundancy of attractiveness judgments are scarce. In one of the first such studies, Rikowski and Grammer (1999) reported a positive relationship between judgments of women's faces and their body odour. They also found a similar association in men's faces and odour, when rated by women in the fertile phase of their menstrual cycle Note that authors assessed cycle phase based on counting methods which appears to be highly unreliable, see Gangestad et al., 2016; Havlíček & Roberts, 2022). Rikowski and Grammer concluded that human faces and body odours provide similar information about mate quality. Several other studies have subsequently reported positive associations between perceived attractiveness of faces and body odours (Mahmut & Stevenson, 2019; Roth, Samara, & Kret, 2021; Thornhill et al., 2003; Thornhill & Gangestad, 1999a), although the strengths of some associations were weak and two other studies (Roberts et al., 2011; Röder, Fink, & Jones, 2013) found no support for this association (see Table S0–6 and Fig. 2). Collectively, the available studies provide some support for both the *backup signals* and *multiple messages* hypotheses.

In view of this, we set out to conduct a systematic review and meta-analysis of the relationship between human body odour and facial attractiveness, to test between the two hypotheses. We collated the published studies and complemented these with unpublished datasets. During this process, we noticed that several of the unpublished datasets that we obtained from researchers also contained ratings of perceived vocal attractiveness. Therefore, we also performed meta-analyses of congruence between body odour and vocal attractiveness. As body odour perception and its relation to other modalities are still somewhat overlooked research topics, we focus our study primarily on the relationships between body odour attractiveness and other sensory modalities. Although of interest, the investigation of the association between facial and vocal attractiveness to a comparable extent (e.g. collecting both published and unpublished evidence) is beyond the scope of the current study.

2. Material and methods

2.1. Systematic review and Meta-analysis

2.1.1. Literature search and study selection

Following the PRISMA 2020 protocol (Page et al., 2021) and PRISMA 2020 checklists (see Supplementary material), we conducted a systematic literature search in July 2020 to identify empirical studies reporting data on the associations between perceived body odour and facial and/or vocal attractiveness. We searched the PubMed and Web of Science (WoS) databases. Topics (WoS) and all fields (PubMed) were searched using the keyword combinations *'odour AND face AND attractiveness', 'odour AND facial AND attractiveness', 'odour AND voice AND attractiveness'* and *'odour AND vocal AND attractiveness'* (WoS search query example TS = (odour) AND TS = (face) AND TS = (attractive); PubMed search query example ((odour[Title/Abstract]) AND (face[Title/Abstract])) AND (attractiveness[Title/Abstract]); results for each query and database are provided in the Supplementary material). Studies were also searched through cross-referencing and by direct correspondence with researchers who had published previously on body odour attractiveness. We contacted 13 authors, 7 of whom responded that they had no suitable data, and 6 of whom provided data.[1] Only articles and research papers written in English were reviewed. Both published and unpublished studies were considered. The complete list of search results is reported in Table S0–5 - Systematic literature search and Prisma Flow diagram (Supplementary material).

2.1.2. Inclusion criteria

A two-step selection process was adopted. First, titles and abstracts of studies identified by the search were screened for inclusion by one team member (VT). Studies were included if they met each of the following criteria: focused on humans (not other species); included ratings of body odour samples and either facial photographs or voice recordings (or both); provided data about perceived body odour attractiveness, and perceived facial and/or vocal attractiveness of the target participants. Second, all entries reporting the relevant data or unclear about reporting the relevant data were screened against the same criteria, where their full texts were examined for suitability. Studies were excluded from the meta-analysis if the key data (perceived body odour and facial or voice attractiveness) were collected but the relevant analyses were not

[1] All authors who provided unpublished data were offered co-authorship of the resulting manuscript. Their involvement in the study is described in the Author Contributions list.

conducted or not reported, unless the authors provided respective effect sizes or raw data for effect size calculations after we contacted them.

We used Pearson's *r* (correlation coefficient) as a measure of the effect size of the association between body odour and facial and/or vocal attractiveness. We excluded studies reporting effect size measures that could not be converted to Pearson's *r* and/or were not available from the authors.

For further details, see the PRISMA 2020 Flow Diagram and Table S0–5 (in the Supplementary material) that contains all selection steps.

2.1.3. Data extraction

Data extracted from the selected studies are reported in Table S0–6 - Summary of published and unpublished data. Two research team members (VT and JTF) individually extracted the data, summarised them, and verified their validity.

2.1.4. Analysis

All statistical tests within this article were performed in jamovi (The jamovi project, 2021). We used the MAJOR (Hamilton, 2021) jamovi module to perform a correlation coefficients meta-analysis, following recommendations by Harrer, Cuijpers, Furukawa, and Ebert (2021). The correlation coefficients of the associations between perceived body odour and facial attractiveness and body odour and vocal attractiveness were converted with Fisher's r-to-z transformation and accompanied by their 95% CI. Fisher's r-to-z transform is the recommended procedure for correcting for bias in studies with small sample sizes (Harrer et al., 2021).[2] Separate meta-analyses were performed for correlations between each pair of stimuli (body odour – facial attractiveness and body odour – vocal attractiveness). We performed each meta-analysis first for both target sexes combined and then separately for each target sex; the results for both sexes combined are reported in the main text, and the results for each sex are provided in the Supplementary material (Table S0–7 - Supplementary Meta-analyses results). We assumed that variation in effect sizes between studies was due to sampling error of true effect sizes or because of other (e.g., methodological) differences between studies. Therefore, we used the random-effects model with a restricted maximum-likelihood estimator (Harrer, Cuijpers, Furukawa, & Ebert, 2021) for heterogeneity statistics (Tau^2). Heterogeneity examines whether variation in the observed correlations results from sampling error. Cochran's Q (which tests whether effect size variability across samples is larger than would be expected by sampling error) and I^2 (which indicates the percentage of variability due to true heterogeneity; I^2 values of 25% are considered low, 50% moderate, and 75% high variability (Higgins, Thompson, Deeks, & Altman, 2003) were computed to quantify the proportion of variance in the observed effects attributable to sampling error (i.e., the extent to which true effect sizes vary within a meta-analysis) (Harrer et al., 2021). In the case of heterogeneity, the meta-analytic results are reported with their 95% prediction intervals (PI). We inspected small-study effects and between-study heterogeneity using contour-enhanced funnel plots and Egger's regression test for funnel plot asymmetry (Harrer et al., 2021); this test was carried out only for the association between perceived body odour and facial attractiveness as its usage is recommended when the number of studies (k) is ≥10 (Harrer et al., 2021; Sterne et al., 2011). To explore potential biases in published vs unpublished effects, we tested the moderator effect and performed separate meta-analyses for published and unpublished effects. Lastly, we also explored the potential moderating effect of the rating design (between- and within-subject design) on observed meta-analytic estimates. These comparisons were carried out only for the association between perceived body odour and facial attractiveness, as both published and unpublished effects were available for this association, and the number of available studies was $k \geq 10$.

2.1.5. Power analysis

We performed analyses of statistical power for the meta-analytic effects in both meta-analyses following Quintana (2015) and Quintana and Tiebel (2019). We conducted a sensitivity analysis to estimate what meta-analytical average effects we have the power to observe with the resulting number of effects per meta-analysis, the average number of stimuli per study (within a given meta-analysis), 5% α and β error rates ($p \leq 0.05$ in two-tailed tests, 1-β error probability ≤0.95 Power), and for potentially low, moderate, and high heterogeneity of the effects (Higgins et al., 2003) (Fig. 1).

2.1.6. Effect size distributions

We calculated effect size distributions (ESD) (e.g., Brydges, 2019; Gignac & Szodorai, 2016; Lovakov & Agadullina, 2021; Nordahl-Hansen, Cogo-Moreira, Panjeh, & Quintana, 2022; Quintana, 2017) for both investigated associations (body odour – facial attractiveness and body odour – vocal attractiveness). Alongside meta-analytic averages, ESD can facilitate more accurate power analyses to determine sample and effect sizes when planning future research in a particular area. The ESD primarily allows for the determination of empirically-based normative guidelines. Thus, instead of Cohen's (1988) traditional 'rule of thumb' conventions for correlations ($r \approx 0.10$: small effect; $r \approx 0.30$: moderate effect; $r \approx 0.50$: large effect), ESD serves as an evidence synthesis derived, field-specific benchmark against which effects from individual studies are compared (e.g., whether the observed effect size in a particular study is smaller, average/medium, or larger than in similar studies). We emphasise that the ESD provides effect size comparison with similar studies but is not designed to quantify the practical significance of observed effects.

To examine the distribution of correlation coefficient effect sizes, we calculated the 50th percentile, representing the average effect size, and the 25th and 75th percentiles, as these are equidistant from the average effect size representing small and larger effects size boundaries, respectively (Cohen, 1992; Quintana, 2017).

2.2. Analysis of the unpublished studies

Ten unpublished datasets (further referred to as Studies 1–10) were secured through personal communication. Data on the association between perceived body odour and facial attractiveness were available in all studies; five studies (Study 2, 5, 6, 7, 10) also included data on voice attractiveness. The Supplementary material contains a detailed description of the methods and results of each study, means per target (Table S0–1 - Means per target), and means per modality (Table S0–2 - Means per modality).

2.2.1. The stability and precision of mean rating estimates

To assess whether the number of ratings for each stimulus type within Studies 1–7, 9, 10 and part of Study 8 provided stable estimates, we calculated the point of stability (POS, a point at which means do not substantially change with additional observations) within a corridor of stability of a mean (COS) (Hehman, Xie, Ofosu, & Nespoli, 2018; Schönbrodt & Perugini, 2013) in R x64 (R Core Team, and Team, 2019) via RStudio (R Core Team, 2021). We used the settings following Hehman et al. (2018): for the 1–7 scale (Studies 1–4, 7, 9), the POS was specified as 95% CI of observed values falling within ±0.5 points (approximately 14%) (Fialová et al., 2020), for the 9-point scale (Study 5, −4 to +4 scale used for odour ratings) within ±0.6 points (∼ 14%), for the 0–1000 scale (Study 6) we set POS at 95% CI within ±70 points (∼ 14%), for the 1–10 scale (Study 8, the replication sample) we set POS

[2] Another approach is to use bias-corrected correlations. In the main paper, we report results using the Fisher's r-to-z transforms. We further ran the two presented meta-analyses with bias-corrected correlations for transparency and comparison between other meta-analyses and their effect size treatments; the analyses are reported in the Supplementary material. Both analyses produced essentially the same results with marginally smaller AIC values for Fisher's r-to-z transformed data.

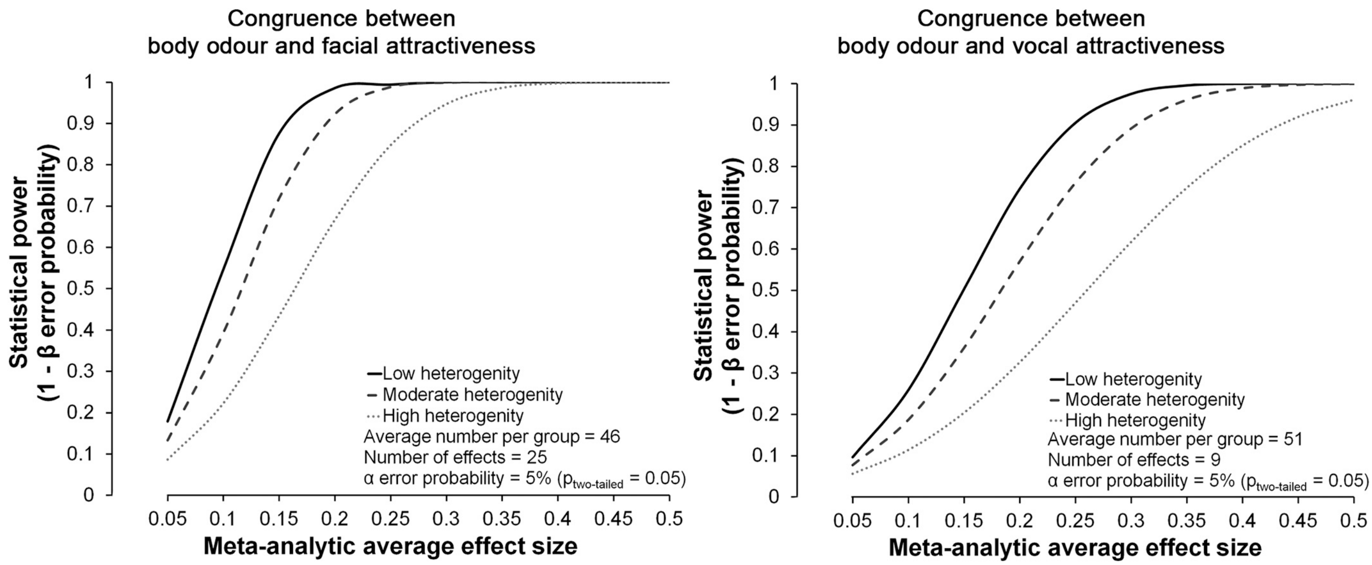

Fig. 1. Power curves for the sensitivity to detect meta-analytic effects as a function of heterogeneity. The plots display the sensitivity analysis for the meta-analysis of congruence between body odour and facial attractiveness (left panel) and between body odour and vocal attractiveness (right panel). Solid, dashed, and dotted curves represent low, moderate, and high heterogeneity. Power curve plots were generated in MS Excel 365 following Quintana *(2015) and* Quintana and Tiebel (2019) and edited in Adobe Photoshop CC2022.

at 95% CI within ±0.7 points (~ 14%) and for the 1–5 scale (Study 10) we set POS at 95% CI within ±0.35 points (~ 14%).

This analysis provided an estimate of the number of raters required to reach predefined POS (and allowed a comparison with the number of raters recruited and an estimation of the size of the raters' pool needed). We further calculated the mean rating precision each study reached with a COS of 95% CI, see Table S0–3 - Point of stability and Intra-class Correlation Coefficients (ICC) in the Supplementary material.

2.2.2. *Assessment of inter-rater reliability*

To assess inter-rater reliability for each stimulus type in Studies 1–7, 9, 10 and part of Study 8, we calculated the ICC (Koo & Li, 2016) using Reliability analysis in the SimplyAgree (version 0.0.2) jamovi module. We used a two-way random model for average agreement (type ICC2k) and followed recommended thresholds for values <0.5 as indicative of poor reliability, values between 0.5 and 0.75 as being of moderate reliability, values between 0.75 and 0.9 indicating good reliability and values >0.9 indicating excellent reliability (Koo & Li, 2016). See Table S0–3 – Point of stability and ICC in the Supplementary material for individual ICC values.

Further, using a linear mixed-effect model, we explored differences in ICCs for different stimulus types. Results are reported in the Supplementary material (ICC comparison).

2.2.3. *Perceptual differences between rating sessions, side-related armpit differences, and an association between short- and long-term attractiveness ratings*

In Studies 1, 2, 5, and 7, ratings were recorded in multiple sessions. To test for potential differences between sessions, we specified linear mixed-effect models. Attractiveness rating (for a specific modality) was set as the dependent variable, the number of sessions as a fixed effect factor, and both the rater and target participants' ID as random effects (example model syntax: Odour attractiveness rating ~ session + (1 | rater ID) + (1 | target ID)).

The raters in Study 5 were presented with the target's body odour samples from both armpits (separately, as two stimuli). Therefore, we used a bivariate correlation analysis (on aggregated ratings per armpit and target participant) to assess the association between the ratings of the two odour samples.

In several studies, body odour (Study 6–1, 6–2), facial (Study 4, 5, 6–1, 6–2, 9), and vocal stimuli (Study 6–1, 6–2) were rated for short- and long-term attractiveness. We used a bivariate correlation analysis (on aggregated ratings per scale type and target participant) to assess the association between these two scales. We initially set $r \geq 0.8$ (Brown, 2006) as the level at which we considered the two attractiveness scales as highly correlated and thus difficult to discriminate. In fact, ratings of short-term and long-term attractiveness were highly positively correlated with all r's ≥ 0.856, thus fulfilling our criteria to consider the two ratings numerically interchangeable. We therefore used the long-term attractiveness ratings for subsequent analyses and labelled these simply as 'attractiveness'.

All linear mixed effect models were run using GAMLj jamovi module (Gallucci, 2021) with REML fit; fixed effect factors were set as 'Simple' contrasts and covariate scaling was set to 'Centred'.

For the individual results, see the Methods and Results of each study in the Supplementary material.

2.2.4. *Association between attractiveness of different modalities*

Previous research reported positive associations between the attractiveness of body odour and facial images (Rikowski & Grammer, 1999; Thornhill et al., 2003; Thornhill & Gangestad, 1999a). Therefore, we ran one-tailed Pearson's r bivariate correlations ($r \geq \rho$) (on aggregated attractiveness ratings per stimulus type and per participant, i.e., the mean rating of a participant was the unit of analysis) between odour and face, and between odour and voice pairs, within each dataset. The resulting correlation coefficients are reported with 95% CI [lower limit, 1].

2.2.5. *Power analysis*

The current study used data from previous studies; therefore, we calculated the sensitivity to detect effects and their critical values for Exact Correlation (Bivariate normal model) using G*Power (Erdfelder, Faul, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007). The parameters were set to a one-tailed test ($r \geq \rho$), sample size (number of targets per individual dataset), 5% α error probability ($p = 0.05$) and

5% β error probability (1-β error probability = 0.95 Power).[3] For the sensitivity of individual studies, including observed effects and the power curves plot, see Table S0–4 - Power analysis, and Fig. S0–1 ibid. in the Supplementary material.

2.3. Data availability and supplementary materials

Datasets, tables of descriptive statistics, detailed descriptions of methods and statistical analyses of individual studies, literature review and meta-analysis methods, and jamovi outputs are all available in the Supplementary material.

3. Results

We extracted 25 effects for the relationship between body odour attractiveness and facial attractiveness, and 9 effects for body odour attractiveness and vocal attractiveness (Table S0–6). These were based on ten unpublished datasets and four published studies describing the association between body odour attractiveness and facial attractiveness, and between body odour attractiveness and vocal attractiveness (from 92 search results, see Table S0–5). The results reported below are based on 1001 target stimuli and 1350 raters.

3.1. Sensitivity to observe meta-analytical effects

With the 25 effects and an average sample size of 46 targets per group in the meta-analysis on the relationship between body odour and facial attractiveness, we reached a sensitivity to observe effects (with 5% α and β error rates) of 0.174, 0.214 and 0.303 for low, moderate, and high heterogeneity, respectively (Fig. 1 – left).

In the case of the meta-analysis on the relationship between body odour and vocal attractiveness, with 9 effects and an average sample size of 51 targets per group, we reached a sensitivity to observe effects (with 5% α and β error rates) of 0.276, 0.339 and 0.484 for low, moderate, and high heterogeneity, respectively (Fig. 1 – right).

Hence, effects smaller than those estimated by our sensitivity analysis would be observed with statistical power below 95%, following the associated curves in Fig. 1. For example, if the meta-analysis on the relationship between body odour and facial attractiveness would have small heterogeneity and observed effects of 0.2, 0.1, or 0.05, it would have ~99%, ~55%, or ~ 17% power to observe them, respectively.

3.2. Association between body odour and facial attractiveness

All 25 effects were included in the meta-analysis on the association between body odour and facial attractiveness. The observed correlation coefficients ranged from −0.436 to 0.867, with the majority of estimates (68%) above zero. The meta-analytical mean showed a statistically significant, weak positive correlation coefficient of 0.104 [0.034, 0.174], Z = 2.93, $p = 0.003$ (Table 1, Figs. 2 and 3). Although Cochran's Q test was not statistically significant, the effect tends to vary across the studies ($Q_{24} = 35.945, p = 0.056$), with small heterogeneity (Quintana & Tiebel, 2019) of about 22% attributable to sampling error. Based on the 95% PI, the *true* outcome is expected to be between −0.069 and 0.277. Results of the Egger's regression suggest no asymmetry in the funnel plot ($\beta_0 = 0.803$, $p = 0.422$, Fig. 3). For female (k = 8) and male (k = 17) targets, the meta-analytical means were 0.163 [0.011, 0.314] and 0.086 [0.005, 0.168], respectively (Table S0–7 - Supplementary meta-analyses results).

3.2.1. Comparison of published and unpublished effects

Considering only the published effects (k = 10), the meta-analytical mean showed a positive correlation coefficient of 0.185 [0.041, 0.328] with a moderate level of heterogeneity (50%). Based on a 95% PI, the *true* outcome thus can be expected between −0.156 and 0.526 (Table 2, Fig. 4). When only the unpublished effects (k = 15) are considered, the meta-analytic mean is 0.052 with 95% CI [−0.024, 0.128] overlapping 0, and 0% heterogeneity (Table 2, Fig. 4). When the publication status (published/unpublished) is used as a moderator, its effect is statistically non-significant (estimate = −0.128 [−0.259, 0.004], $p = 0.057$, heterogeneity $I^2 = 10.25\%$).

3.2.2. The effect of rating design

For studies (k = 16) using a between-subject rating design (different groups of participants provide attractiveness ratings for different stimulus types), the meta-analytical mean estimate for body odour and facial attractiveness was 0.089 with 95% CI [−0.05, 0.183] overlapping zero ($I^2 = 38.29\%$). Studies (k = 9) using a within-subject rating design (each participant judged both stimulus types) also showed a weak positive association between the modalities, 0.146 [0.036, 0.256] ($I^2 = 0\%$), (Table 3). When the rating design was used as moderator, its effect is statistically non-significant (estimate = −0.034 [−0.201, 0.134], $p = 0.692$, $I^2 = 0\%$), Table 3.

3.3. Association between body odour and vocal attractiveness

The association between body odour and vocal attractiveness (k = 9) was weakly positive and statistically significant. The observed correlation coefficients ranged from −0.189 to 0.297, with the majority of estimates (89%) above zero. The meta-analytical mean estimate was 0.098 [0.004, 0.192] with Z = 2.038, $p = 0.041$ (Table 1, Figs. 2 and 3). Cochran's Q ($Q_8 = 4.8, p = 0.779$) indicated that the effect did not vary between studies, with 0% of the observed effect attributable to sampling error. Considering females and males separately, the meta-analytical means were 0.143 [0.024, 0.263] for female targets (k = 5) and 0.024 [−0.128, 0.177] for male targets (k = 4) (Table S0–7 - Supplementary Meta-analyses results).

3.4. Effect size distributions

We constructed effect size distributions from all available effect sizes for the association between body odour and facial attractiveness (n = 25) and the association between body odour and vocal attractiveness (n = 9). In both cases, the 50th percentile values (average/medium effect size) are ~0.1 and equal to the meta-analytic averages (~ 0.1), the 25th percentile (small/below average effect size boundary) values are ~0, and the 75th percentile (above average/large effect size boundary) values are ~0.2. The distributions and percentiles for small (25th), medium (50th, median), and large (75th) effect sizes are presented in Fig. 5 and Table 4.

4. Discussion

Our results indicate that, although the association between body odour attractiveness and facial attractiveness is positive, the summary effect is relatively small ($r \sim 0.1$). We observed similar patterns and magnitudes of effects for female and male targets and also for the odour-voice attractiveness association. We suggest that body odour may provide distinct and non-redundant information about an individual's mating-related qualities compared to that available within either facial or vocal cues. Thus, concerning perceived attractiveness, body odour may provide different and non-redundant cues to an individual's mating-related qualities compared to cues communicated through the face and voice.

These findings contrast with those of Rikowski and Grammer (1999), who observed a strong positive correlation ($r_{19} = 0.7$) between facial

[3] We decided to choose a 1:1 ratio of the Type I and II error rates for all performed analyses, as we see committing both errors as of equal significance in this instance.

Table 1
Meta-analysis and heterogeneity results.

Congruence in	k	Estimate (Fisher's z)	95% CI		p	95% PI	
			LL	UL		LL	UL
Body odour and Facial attractiveness	25	0.104	0.034	0.174	0.003	−0.069	0.277
Body odour and Vocal attractiveness	9	0.098	0.004	0.192	0.042		

Heterogeneity Statistics	Tau	Tau^2	I^2 (%)	H^2	Q	df	p
Body odour and Facial attractiveness	0.079	0.0062	20.84	1.263	35.696	24	0.059
Body odour and Vocal attractiveness	0	0	0	1	4.8	8	0.779

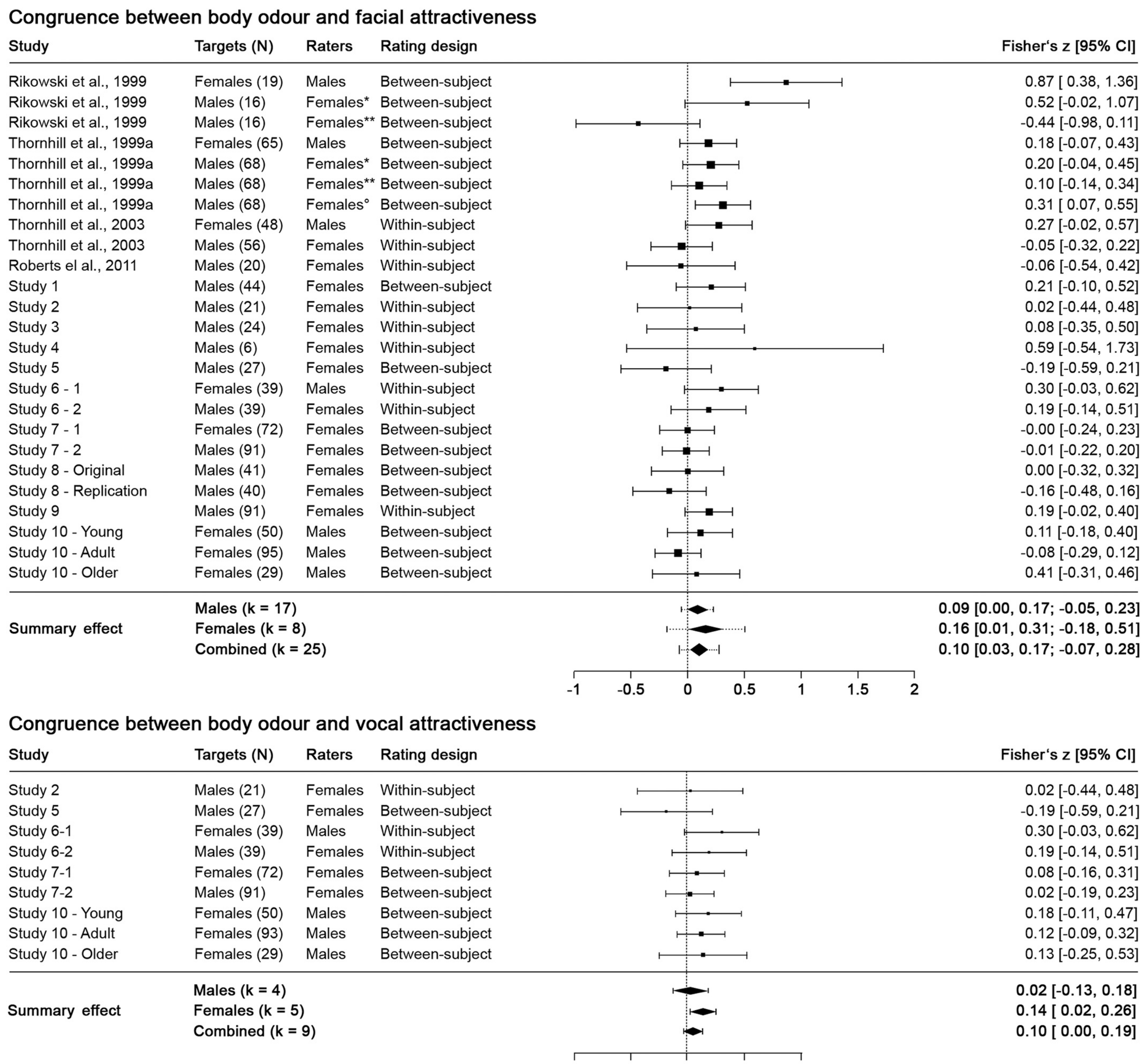

Fig. 2. Forest plots for congruence meta-analyses. Squares represent weighted mean effects of individual studies, and error bars their 95% confidence intervals. Diamonds represent summary effects, their width the 95% CIs, and dashed error bars their 95% PIs. *Female raters in fertile, **non-fertile phase of their menstrual cycle, and °hormonal contraception users. Summary effects are reported in Fisher's z-transformed correlation coefficients with 95% confidence intervals and in heterogeneous effects also followed with 95% prediction intervals. Forest plots were generated in jamovi, and edited in Adobe Photoshop CC2022.

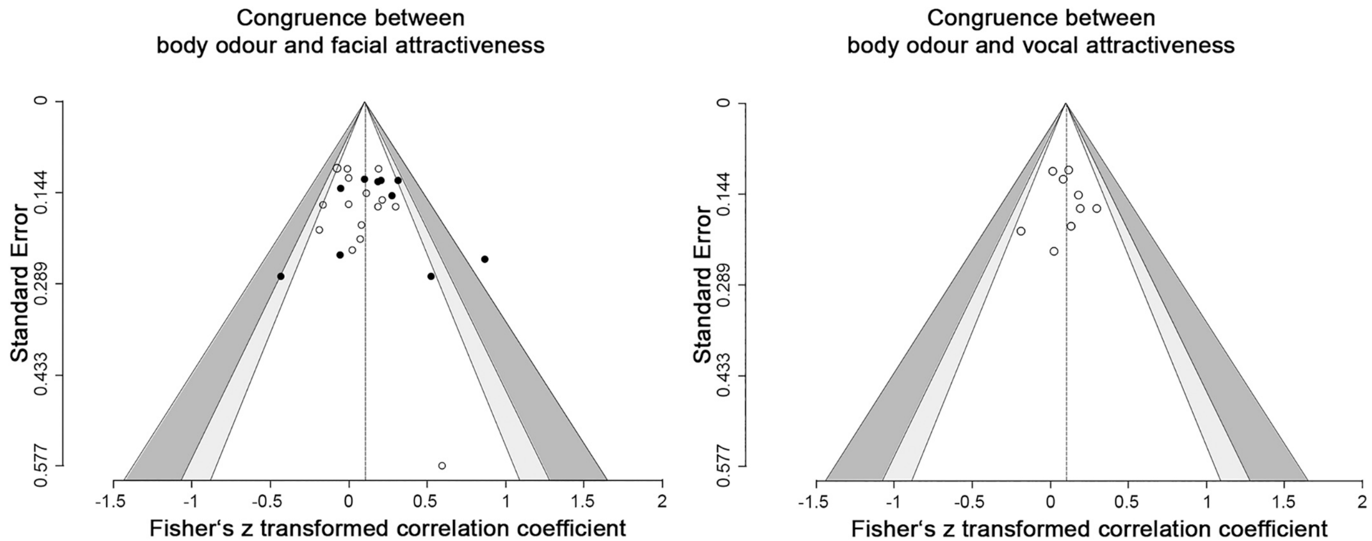

Fig. 3. Funnel plots for congruence meta-analyses. Area outside the contour-enhanced funnels represent *p* values <0.01, dark grey areas p values between 0.01 and 0.05, light grey p values between 0.05 and 0.1, and areas inside the funnel p values >0.1. Full circles illustrate published and empty circles unpublished studies. Dashed line show summary effect sizes; Y-axis is the standard error of Fisher‘s z. Funnel plots were generated in jamovi, and edited in Adobe Photoshop CC2022.

Table 2
Meta-analysis and heterogeneity results for published and unpublished effects.

Origin	k	Estimate (Fisher's z)	95% CI		p	95% PI	
			LL	UL		LL	UL
Published effects	10	0.185	0.041	0.328	0.012	−0.156	0.526
Unpublished effects	15	0.052	−0.024	0.128	0.182		
Moderator		−0.128	−0.259	0.004	0.057		

Heterogeneity Statistics	Tau	Tau^2	I^2 (%)	H^2	Q	df	p
Published effects	0.158	0.0249	49.91	1.996	19.813	9	0.019
Unpublished effects	0	0	0	1	11.92	14	0.613
Moderator	0.052	0.0027	10.25	1.114	31.733	24	0.106

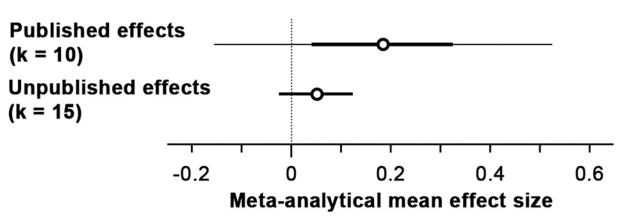

Fig. 4. Comparison of meta-analytic averages between published and unpublished effects. Circles represent mean effects. Thick error bars their 95% CI and thin error bars 95% PI. Due to observed heterogeneity only in the published effects, the mean effect is accompanied by 95% PI. The plot was generated in Adobe Photoshop CC2022.

and body odour attractiveness, but concur with more recent studies (Roth et al., 2021) that report a weak association between body odour, facial, and vocal attractiveness. Similarly, our findings are in line with those of two studies (Mahmut & Stevenson, 2019; Roth et al., 2021) that did not meet our formal inclusion criteria due to their non-parametric and non-frequentist data analysis (Table S0–5). In a sample of 82 female raters and 91 male donors, Mahmut and Stevenson (2019) reported Spearman's $\rho = 0.3$ for the association between body odour and facial sexiness. Using Bayesian analysis with a sample of 70 participants who served as both donors and raters, Roth et al. (2021), reported that body odour, facial, and vocal attractiveness were positively correlated but with small effect sizes. It is worth noting, however, that the authors discuss their findings of small and positive effects in favour of the *backup signals* hypothesis; we would disagree with this interpretation. The shared variability of attractiveness ratings resulting from the summary effects across the two pairs of modalities in the present meta-analyses was <1%, suggesting minimal (if any) redundancy in information transferred through these modalities.

In studies concerning an association between facial and vocal attractiveness, the current evidence shows inconsistent results, ranging from strong positive correlations in women only (Abend, Pflüger, Koppensteiner, Coquerelle, & Grammer, 2015; Collins & Missing, 2003; Wheatley et al., 2014) to weak (Zuckerman, Miyake, & Elkin, 1995) or no significant associations (Zäske et al., 2020). This range suggests that the overall pattern of relationships might be similar to that found in the present study between odour and these other modalities. However, there is currently no systematic investigation or meta-analysis available for the association between facial and vocal attractiveness to our best knowledge.

4.1. Notes on the meta-analyses and renumber other heading

Notes on the meta-analyses Although Fig. 4 shows a stronger (over 3×) positive mean effect for published effects than unpublished ones, but the meta-analytical mean of unpublished effects provides a more precise estimate: the mean effect (and over half of its 95% CI) falls within the 95% CI (and entirely within 95% PI) of the published effects. If the present study were based only on published evidence, it would

Table 3
Meta-analysis and heterogeneity results for between- and within-subject rating design.

Rating Design	k	Estimate (Fisher's z)	95% CI		p	95% PI	
			LL	UL		LL	UL
Between-subject	16	0.089	−0.05	0.183	0.062	−0.155	0.334
Within-subject	9	0.146	0.036	0.256	0.009		
Moderator		−0.034	−0.201	0.134	0.692		

Heterogeneity Statistics	Tau	Tau^2	I^2 (%)	H^2	Q	df	p
Between-subject	0.115	0.0133	38.29	1.62	29.439	15	0.014
Within-subject	0	0	0	1	5.605	8	0.691
Moderator	0.087	0.0076	24.52	1.325	35.708	24	0.044

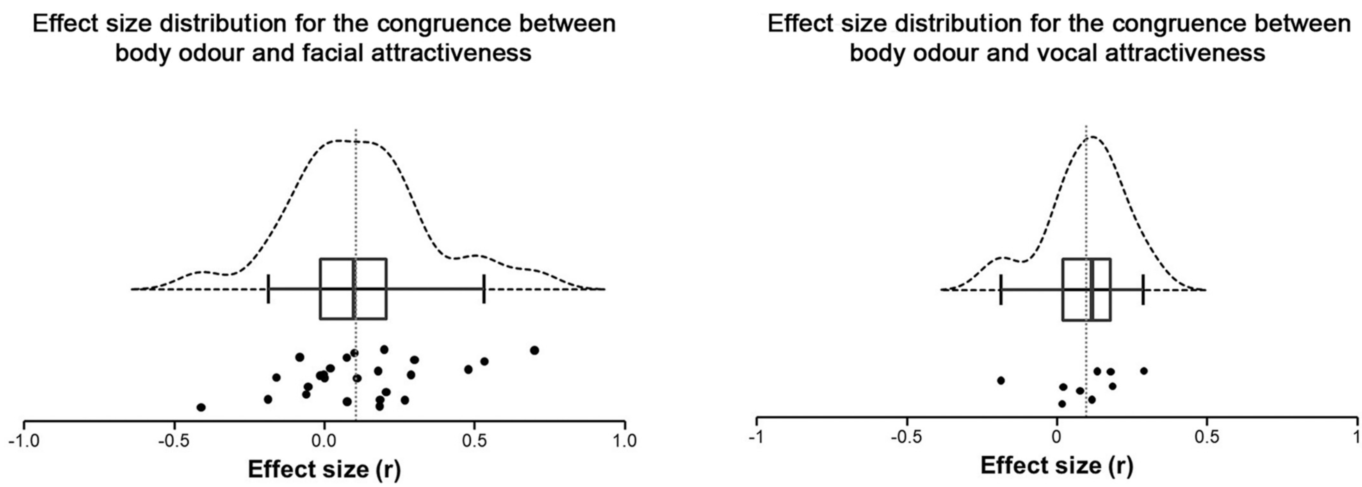

Fig. 5. Raincloud plots for effect size distribution. Density plots show effect sizes distribution, boxplots show median (thick line), 25th and 75th percentile (interquartile range, box), and minimum and maximum (error bars); jittered dots represent individual effect sizes; dotted vertical line shows effect size average for each meta-analysis (left 0.104, right 0.098). Raincloud plots were generated in JASP (0.16.2) and edited in Adobe Photoshop CC2022.

Table 4
Effect size distributions.

Congruence in	Number of effects	Percentiles		
		25th	50th	75th
Body odour and Facial attractiveness	25	−0.013	0.1	0.206
Body odour and Vocal attractiveness	9	0.02	0.116	0.178

thus report a stronger and less precise estimate of the meta-analytic effect for associations between assessments of body odour and facial attractiveness. Moreover, a meta-analysis of body odour and vocal attractiveness would not be possible as the literature search identified only a single study fulfilling the inclusion criteria (Roth et al., 2021 discussed above). This highlights the importance of considering unpublished data in quantifying effects through systematic reviews and evidence synthesis.

Although we generally observed low levels of heterogeneity in our meta-analyses, they rely on a relatively small number of effects and the sensitivity of our analyses is correspondingly low. In addition, the statistical power in many of the available studies is low, due to a relatively small number of stimuli (Table S0–4). The average number of raters per stimuli (mostly body odour stimuli) often resulted in wider corridors of rating stability (Hehman, Xie, Ofosu, & Nespoli, 2018) and thus less precise estimates of mean ratings (Table S0–3). This mainly arises from logistical limitations related to procedures employed in body odour sampling and rating. In contrast to facial images and vocal recordings, body odour stimuli can be used only a limited number of times due to microbial transformation and signal degradation (Lenochová, Roberts, & Havlíček, 2009). Furthermore, the number of odour stimuli that one rater can assess within a reasonable time is limited by olfactory adaptation (Köster & de Wijk, 1991). These issues hinder the accuracy of the present findings and represent challenges for further research.

In addition to the meta-analytical results, the current article presents a systematic overview of studies conducted over the last two decades, including data collection methods, sample sizes, populations, and observed ratings (Tables S0–6). We also included observed effect size distributions showing that commonly used correlation thresholds overestimate effect sizes observed in studies, where average and larger-than-average effects (50th and 75th percentile, respectively) are 'only' ~0.1 and ~ 0.2. Based on the unpublished datasets, where more detailed insight can be provided, the average number of stimuli used in this type of research is ~46 giving us sensitivity to observe correlations ≥0.49 (with 0.05 $p_{\text{two-tailed}}$ and 95% power, ≥ 0.39 with 80% power). On average, in these studies, body odour, and facial and vocal stimuli are rated for attractiveness by ~25, 31, and 32 raters, respectively, though based on our corridor of stability analysis samples ≥35 seem to be needed for more precise estimates. Overall, all three stimulus types seem to be rated with good reliability (mean ICC2k ~0.8), and we found no differences in reliability between stimulus types. See Tables S0–1, 2, 3 and 4, and ICC comparison in the Supplemental materials for further details. Future research investigating the association in attractiveness rating between modalities could benefit from this systematic overview, including effect size distributions, to plan and convey magnitudes of observed effects in comparison to the body of up-to-date literature.

4.2. Alternative reasons for the observed effects

It is conceivable that the associations between individual modalities are underestimated because (a) studies use 'snapshots' of an individual which might provide only a rough estimate of his or her mating-related qualities, and (b) these snapshots vary in duration across modalities. Odour stimuli are typically collected over a longer period (12-24 h) and may, therefore, provide a more reliable quality estimate. In contrast, vocal stimuli often last <1 min. and visual images capture less than a second. Previous studies testing the association between body odour attractiveness and physical attractiveness assessed from videos found a stronger correlation ($r = 0.32$) compared to the association between body odour attractiveness and facial attractiveness ($r = -0.08$) (Roberts et al., 2011). Thus, sampling time might influence the reliability of mating-related quality estimates. A reviewer also argued that the reason for the weak correlation between odour attractiveness and the two other modalities could be higher variability in ratings of body odour, perhaps because it is considered that olfactory judgments are either more difficult or more subjective. However, our ICC analysis shows that the level of agreement is comparable across the three modalities.

Similarly, the weak correlations that we observe between attractiveness assessments of different stimulus types might result from experimental (laboratory-based) settings and some variations in protocols. These include, for example, control over facial expressions during image acquisition, the volume of voice recordings, and dietary restrictions in body odour sampling. Although methodologically challenging, the use of more naturalistic stimuli with facial expressiveness, the prosody of speech and natural variation in body odour (Roberts et al., 2022) may provide additional insight into the patterns of associations and congruence across sensory modalities investigated here.

Further, earlier studies reporting positive associations between attractiveness and putative markers of mating-related quality had failed to replicate, especially when they were based on small samples. Many studies that were included in the current analysis had different groups of participants providing attractiveness ratings of the stimulus types (between-subject rating design). A high inter-individual variation in attractiveness ratings in some modalities would lead to a weak correlation between the modalities because the target is rated by some people in one modality and by other people in the other. Studies using a design where each participant judged all stimulus types (within-subject rating design) also tend to show a weak correlation between the modalities, meaning that weak correlations in individual studies cannot be solely due to study design.

An individual's mating-related quality may be perceived more accurately by combining cues from different modalities that independently correlate with mate preferences. However, most studies on physical attractiveness examine the influence of individual modalities separately, a design that lacks ecological validity because, in everyday life, we perceive others through multiple senses simultaneously (Groyecka et al., 2017). Similarly, the present meta-analysis is based on studies investigating several modalities separately, not on multimodal perception, which is a result of simultaneous perception across different sensory modalities. The resulting perception can differ qualitatively from the sum of the properties of its components and convey a unique message, or one modality can affect information transmitted by the other modalities, being different from the *backup*and *multiple messages*concepts (Halfwerk et al., 2019; Mitoyen, Cliodhna, & Leonida, 2019). How information based on different modalities contributes to overall attractiveness judgments is poorly understood (e.g., Ferdenzi, Delplanque, Atanassova, & Sander, 2016). Current research into the integration of human mate preferences indicates that they are best described by the Euclidean model (Conroy-Beam et al., 2019). Whether a similar pattern of integration can be expected in the case of physical attractiveness or whether it would follow another form, as explained by additive or threshold models, remains to be investigated (Csajbók, Bérkics, & Havlíček, 2022; Havlíček, Štěrbová, & Csajbók, 2022).

4.3. Theoretical implications

It has been proposed that attractiveness reflects an individual's mating-related qualities (e.g., in terms of health and fertility). Perceived facial attractiveness is influenced by several features, including symmetry, prototypicality, sexual dimorphism, adiposity, and skin condition. For instance, prototypicality is thought to be a marker of heterozygosity, symmetry a marker of developmental stability, while sexual dimorphism is a marker of sex hormone levels and skin quality is a marker of health status (for review, see Stephen & Luoto, 2022). Similarly, it has been suggested that body odour may also provide information about heterozygosity, developmental stability, sex hormones and health (for review, see Havlíček, Fialová, & Roberts, 2017). Hence, one might expect at least moderate associations between the attractiveness of these modalities, but we found only weak associations. Several associations between attractiveness and the proposed underlying qualities were recently revisited (Stephen & Luoto, 2022) and others are still debated. These include links between hormonal profiles and facial attractiveness (Jones, Jones, Shiramizu, & Anderson, 2021) or between body odour attractiveness and MHC heterozygosity (Havlíček, Winternitz, & Roberts, 2020).

Visual, olfactory, and acoustic modalities may provide unique (and non-redundant) information about an individual's mating-related quality. Our results are in line with the *multiple messages* hypothesis but seem to provide little support for the *backup signals* hypothesis. Moreover, they correspond with the majority of animal studies that have reported multiple traits to be unrelated, suggesting that backup signals are less common than multiple messages (Badyaev, Etges, Faust, & Martin, 1998; Candolin, 2003; Kraak, 1999). We speculate that facial appearance primarily provides cues to more stable characteristics such as the development of hormone-related secondary sexual characteristics and maturation (Marečková et al., 2011; Whitehouse et al., 2015). In contrast, body odour may provide cues to more variable characteristics, such as current health (Olsson et al., 2014; Sarolidou et al., 2020) and fertility status (Gildersleeve, Haselton, Larson, & Pillsworth, 2012; Havlíček, Dvořákova, Bartoš, & Flegr, 2006). These are provocative and open questions that require in-depth investigations.

In conclusion, the present study found weak congruence between attractiveness assessments of human body odours and those of faces or voices. These results provide little support for the *backup signals* hypothesis in explaining the use of multiple modalities in attractiveness assessments, but favour the *multiple messages* hypothesis, suggesting that body odour provides information about mating-related quality different from that of faces or voices.

Ethics

All procedures within the individual studies were carried out following the Declaration of Helsinki, and Institutional Review Boards approved each study. Individual approvals can be found in the Supplemental Materials.

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.evolhumbehav.2022.11.001.

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11 CONCLUSIONS

The thesis consisted of two main parts. The first part provided an introduction to the topic of visual attention and perception of faces in intersexual and intrasexual selection and outlined the theoretical background for the five empirical studies that were presented in the second part of the thesis.

The first part of the thesis started with a brief introduction to the mechanisms of intersexual and intrasexual selection. Furthermore, facial attractiveness and dominance were discussed, as well as the qualities to which they are proposed to cue. We showed which facial features (not only eyes, nose, and mouth but also, e.g., cheeks and chin) should be important in judgements of facial attractiveness and dominance or formidability, though we also discussed that direct visual attention is not always investigated to corroborate their further importance. Eye-tracking methods were briefly introduced as one way to assess unconscious automatic visual attention processes. The results of some previous eye-tracking studies investigating visual attention towards faces, especially when judging attractiveness or dominance, were discussed. We showed that eyes, nose, and mouth receive the most visual attention, and that is across sexes and contexts/judgements. Moreover, it seems that eye-tracking studies focusing specifically on contexts relevant to intersexual and intrasexual selection, and especially combining both in one investigation, are mostly missing. Further, we briefly inspected the relationship between individual modalities in the assessment of attractive individuals. We introduced two main competing hypotheses, which discuss whether information about conspecifics gained from individual modalities overlaps or whether each modality provides distinct information about facets of an individual's quality.

The second part of this thesis starts with a study that tested the relationship between facial attractiveness, healthiness, facial colouration, and reactivity of the immune system induced by vaccination against hepatitis A/B and meningococcus. This is due to an extensive body of literature connecting facial attractiveness with the effective immune system or health, though often not investigating the relationship with direct measures. Contrary to our expectations, we found no associations between antibody levels induced by vaccination and perceived characteristics, but also facial colouration (Pátková et al., 2022). Though found by some (Rantala et al., 2012), recent studies often tend to fail to find a relationship between facial appearance and immunocompetence; for summary, see Jones et al. (2021). It is possible that the quality of the immune system is not directly perceivable through facial characteristics, or measures of the immune system used in the studies were unable to capture the immune system function in its entirety. It is also possible that studies measured different components of the immune system than those that indeed are related to facial attractiveness. Future studies might try to employ more comprehensive measures of the immune system and focus on acquiring larger sample sizes. Moreover, another alternative explanation for the null results might be that our sample (and many of the others, e.g., Cai et al., 2019; Foo et al., 2017) come from countries with relatively good medical care under which the differences in immune function might not be manifested (and influence facial appearance). Lastly, it has been proposed that facial attractiveness might not actually cue immunocompetence (Jones et al., 2021), which is why some researchers propose that face research could focus more on qualities connected with an individual's lifestyle, such as exercise or diet to which facial appearance might cue, which can also promote more immediate changes in appearance. More immediate changes in appearance can be, however, caused also by acute illness.

In the second study, we focused on changes in facial appearance induced by immune system activation by vaccination, which simulated the state of acute illness. We found that the faces of individuals with activated immune systems were perceived as less attractive and healthy, while their body odour was perceived as more attractive (Schwambergová et al., in revision). This corresponds with other studies showing that individuals are sensitive to illness cues in others (Axelsson et al., 2018; Regenbogen et al., 2017). One of the main limitations of this study was undoubtedly the smaller sample size, and future studies should strive for larger ones. Moreover, future research might employ repeated stimuli collections in intervals after activating the immune system to shed more light on how long the changes in body odour and faces prevail.

Specific facial features were proposed to be important for judgements of attractiveness and dominance or formidability, but direct visual attention to them hasn't been thoroughly investigated. The third, eye-tracking, study presented the results of the investigation inspecting visual attention to faces and their features under the contexts of intersexual and intrasexual selection, i.e., assessment of a potential partner and rival. It showed that women gave more visual attention to partners than rivals but also that women gave more attention to faces under the two contexts than men. Moreover, despite numerous perception and GMM studies deeming specific facial features important for judgements of attractiveness and dominance (e.g., Cunningham, 1986; Třebický et al., 2013; Windhager et al., 2011), this study showed that by far, most visual attention was attracted by eyes, nose and mouth. Slight variations in visual attention have been found between contexts and sexes for features such as cheek and chin, but the amount of direct visual attention towards these features was small. One of the limitations of this study is that although it included male and female targets and raters, only opposite-sex attractiveness assessment and same-sex assessment of dominance were made, and it would be beneficial to investigate same-sex assessment of attractiveness and opposite-sex assessment of dominance with the same design.

Prior research suggested that the level of stimuli's attractiveness or formidability might be important in visual attention to faces. The fourth, eye-tracking, study investigated whether more attractive and formidable faces will attract more visual attention and how will be the visual attention distributed among facial features. The visual attention to faces with varying degrees of formidability hasn't been previously investigated. Contrary to previous studies (Leder et al., 2016; Mitrovic et al., 2018), we showed no relationship between the amount of rater's visual attention and the level of the target's facial attractiveness. However, we showed that faces with a medium than a high level of formidability received more visual attention. Regardless of the level of facial attractiveness or formidability of the targets, eyes, nose, and mouth received the most visual attention. As in our previous study (Pátková et al., in revision), we found variations in visual attention towards, e.g., the chin, here in relation to rater's sex and the target's level of attractiveness and formidability. However, the visual attention towards these areas was, in comparison to the eyes, nose, and mouth regions, small, and we discussed its real impact.

Our eye-tracking studies provide limited evidence for highlighting the importance of features such as jaw, chin or cheekbones, to name a few, in judgements relevant to intersexual and intrasexual selection, as proposed by GMM (Mitteroecker et al., 2015; Třebický et al., 2013; Windhager et al., 2011) and perception studies (Cunningham, 1986; Cunningham et al., 1990; Keating, 1985). However, in the manuscripts, we also discuss that while direct attention to these features might not be given, people might still be able to see them and process the information they contain due to the area the central vision covers. More research into the topic is needed, and we would promote using the highest quality and ideally life-sized images in future studies, as it enables us to focus on visual attention to faces in settings that resemble real-life conditions. We further stress the careful consideration of the AOIs.

Lastly, relying on a single modality to assess individuals' attractiveness might lead to an error, and people mostly assess the attractiveness of others based on multiple sensory modalities. Therefore, the fifth study investigated the congruence between olfactory and visual and olfactory and vocal modality in attractiveness assessment. The study, using systematic review and meta-analysis, showed that human body odour and facial attractiveness are positively associated, same as body odour and vocal attractiveness, but the magnitude of the relationships is weak and therefore provides support for the “multiple messages” hypothesis.

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The supervisor (and co-author) signed below agrees with submitting this article as part of this PhD thesis.

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