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Mgr. Dagmar Schwambergová

OLFACTION-MEDIATED BEHAVIOURAL DEFENCE MECHANISMS IN HUMANS

ČICHOVÝ BEHAVIORÁLNÍ OBRANNÝ SYSTÉM U ČLOVĚKA

Doctoral thesis

Supervisor: doc. Mgr. Jan Havlíček, Ph.D.
Advisor: Mgr. Jitka Třebická Fialová, Ph.D.

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DECLARATION

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Mgr. Dagmar Schwambergová

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"Smell is a potent wizard that transports you across thousands of miles and all the years you have lived."

~ Helen Keller

ABSTRACT

This thesis consists of two parts. The first part introduces the importance of behavioural defence mechanisms, specifically the behavioural immune system and mainly in humans. I review current knowledge regarding behavioural defence mediated by odour cues. Although behavioural defence mechanisms are important for all individuals who live in social groups, I focus on mate choice, because that is the context in which olfaction-mediated behavioural defence is studied the most. Subsequently, the importance of olfaction is demonstrated using the example of self-inspection and I discuss how the behavioural immune system may be intertwined with this relatively understudied behaviour. Finally, last chapter deals with associations between olfaction and other modalities that play a role in the detection of cues which help select a healthy and immunocompetent partner.

In the second part of the thesis, I present nine papers: three reviews and six empirical studies. The review papers summarise the functioning of the behavioural immune system, olfaction-mediated pathogen avoidance in mammals, and the merely weak association between attractiveness ratings based on different modalities. The first empirical paper investigates whether the threat caused by the Covid-19 pandemic led to increased perceived disgust, which is an important variable linked to the functioning of the behavioural immune system. We found increased levels of moral disgust during the first wave of the pandemic, but the effect was not observed in the domain of pathogen disgust. The following paper focuses on changes in the attractiveness of body odour, face, and voice after immunoactivation by vaccination. We found that after vaccination, perceived body odour attractiveness increased, while perceived facial attractiveness decreased. The third empirical study explores associations between body odour quality and the functioning of the immune system. We found no significant association between antibody levels induced by vaccination and perceived body odour attractiveness, and no association between the basal key parameters (innate and adaptive) of the immune system and body odour quality. A similar outcome was found for faces in the following study, contrary to previous evidence where the association was reported in men. The fifth empirical study was qualitative, and its aim was to map the importance of body odour for women in intimate and sexual encounters. The results indicated that women's odour perception was often context-dependent and negatively modified by partner's specific odour (after workday, workout, or when the partner is ill) during intimate encounters. Finally, the last paper focuses on olfactory self-inspection and possible functions of this behaviour. For instance, individuals who reported

more frequent health issues sniff themselves more frequently in areas such as the armpits, feet, or their breath, probably to check for possible changes in smell due to illness.

KEYWORDS
Behavioural immune system, olfaction, health status, pathogens, immune system

ABSTRAKT

Předkládaná disertační práce se skládá ze dvou částí. První část představuje behaviorální obranné mechanismy u člověka, zejména behaviorální imunitní systém. V této části také shrnuji poznatky o behaviorální obraně zprostředkované čichovými vodítky. Přestože jsou zmíněné obranné mechanismy důležité pro všechny jedince žijící v sociálních skupinách, disertační práce na toto téma nahlíží v kontextu výběru partnera, neboť je tento kontext nejvíce studovaným. V další kapitole pak poukazuji na důležitost čichové sebe-inspekce, a jak toto dosud jen velmi málo studované chování může být propojeno s behaviorálním imunitním systémem. Závěr první části práce je věnován vztahu mezi čichem a dalšími modalitami, které přispívají k rozpoznání zdravého a imunokompetentního partnera.

V druhé části disertační práce představuji celkem devět článků, z čehož jsou tři přehledové články a šest empirických studií. Přehledové články shrnují fungování behaviorálního imunitního systému, čichem zprostředkovaného vyhýbání se patogenům a jak jsou vztahy mezi hodnoceními atraktivity z různých modalit asociovány jen slabě. První empirický článek se poté zabývá tím, zdali hrozba vyvolaná koronavirovou pandemií vede ke zvýšení vnímaného znechucení, jakožto hlavní proměnné asociované s behaviorálním imunitním systémem. Zjistili jsme, že dochází ke zvýšení morálního znechucení v průběhu první vlny pandemie, avšak stejný efekt nebyl pozorován u klíčové patogenní domény. Další článek se zaměřil na změny v atraktivitě tělesné vůně, tváří a hlasů po očkování. Zde výsledky ukázaly, že došlo ke zvýšení hodnocené atraktivity tělesné vůně po očkování v porovnání s tvářovou atraktivitou, u níž došlo naopak ke snížení. V třetí empirické studii nás zajímal vztah mezi kvalitou tělesné vůně a fungováním imunitního systému. Nenašli jsme však žádnou signifikantní asociaci mezi nárůstem protilátek po očkování a vnímanou atraktivitou, stejně tak jsme nenašli souvislost mezi klíčovými parametry (nespecifickými a specifickými) imunitního systému a hodnocenou kvalitou tělesné vůně. Stejný výsledek nám ukázala další studie, kde jsme se zaměřili na atraktivitu tváří, přestože předchozí evidence poukazovala na pozitivní vztah mezi hodnocenou atraktivitou a nárůstem protilátek u mužů. Pátá empirická studie byla kvalitativní a pomohla nám zmapovat důležitost tělesné vůně v intimních a sexuálních situacích. Výsledky poukázaly na to, že percepce tělesné vůně u žen je závislá na kontextu a je negativně modulovaná specifickou vůní (po práci, cvičení, nebo když je partner nemocný) během intimních situací. Poslední článek se zaměřuje na čichovou sebe-inspekci a možné funkce tohoto chování. Například jedinci, kteří reportovali častější zdravotní obtíže, tak si také častěji čichali k

oblastem jako je podpaží, nohy nebo dech; pravděpodobně kvůli kontrole možné změny tělesné vůně v důsledku nemoci.

KLÍČOVÁ SLOVA
Behaviorální imunitní systém, čich, zdravotní stav, patogeny, imunitní systém

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PART I.

INTRODUCTION

Life in social groups at higher population densities brings various benefits and risks. Sociality can benefit individuals by presenting better and more diverse mating opportunities, greater protection from predators, or sustained access to resources (Silk, 2007). On the other side, social species are more vulnerable to infectious diseases because they have higher contact rates and live in close proximity, which benefits the pathogens (Altizer et al., 2003). In addition to the physiological immune system, which is responsible for organisms' active defence against pathogens, the behaviour of individuals can also significantly contribute to infection avoidance. Such behavioural defence mechanisms assume perceptual sensitivity to markers of health status in conspecifics, because only then can individuals engage in possible behavioural avoidance. The mechanism responsible for this avoidance is known as the behavioural immune system. In the past decade, it has been extensively studied in humans (Schaller and Park 2011, Bradshaw and Gassen, 2021), but seems to exist in non-human mammals as well (Kavaliers and Choleris 2018).

Unlike other threats, pathogens usually cannot be directly perceived by our senses. Individuals must therefore rely on cues linked to pathogen activity, such as disgusting smell, visibly decaying meat, the sound of someone vomiting, etc. Possible pathogen source can be identified by each of the senses. In most mammalian species, olfaction clearly plays a role in chemical communication between conspecifics. A number of studies showed that female mice prefer the urine odour of noninfected males over the urine odour of males infected with various endo- or ectoparasites (e.g., Kavaliers and Colwell 1995a, Kavaliers et al. 1997, Zala et al. 2004, Willis and Poulin 2000, Kavaliers et al. 2003). In humans, olfaction has been underestimated and most studies focused on vision, but a body of evidence accumulated over the past several decades indicates that olfaction plays an important role for humans as well. It is also important in chemical communication: olfaction helps establish a mutual relationship between a mother and her newborn baby (Schaal, 2017) and it plays an important role in mate choice. By smell, individuals can obtain various information about their conspecifics; they can assess the genetic quality of potential partners (Thornhill and Gangestad, 1999) or the current health status of other people (Olsson et al., 2014).

Although most research on the detection of body odour from infected individuals took place in the context of mate choice, the ability to distinguish between healthy and infected individuals

is important also with respect to other conspecifics. Any direct contact, for example grooming or (in human case) hugging, can lead to pathogen transmission and potential health risk.

This thesis investigates olfaction-mediated defence mechanisms in humans. The first part serves as an overview which outlines and elaborates the context within which the empirical studies and review papers from the second part of the thesis are then set. Because the subject is at length discussed in the papers, at this point I just go over the papers briefly in order to highlight some future directions and problematic parts of this topic.

In **Chapter 1,** I focus mainly on the importance of the behavioural immune system as a repertoire of defence mechanisms which function alongside the physiological immune system and outline how this system works. **Chapter 2** introduces the most common methodological approaches to testing the behavioural immune system with emphasis on the system's functioning outside of the laboratory during Covid pandemic. The following **Chapter 3** deals with the role of olfaction in behavioural defence, because this modality may help perceivers maintain safe distance, thus avoiding any direct contact with the potential source of contagion. In this chapter, it is noted that behavioural defence mechanisms are important for all socially living organisms, but since most studies test the issue in the context of mate choice, we shall also at some point investigate partner selection. **Chapter 4** points to the possibility of humans acquiring various information about themselves from their own body odour. It has been established that we can assess the health status, diet, emotional state, gender, age etc. from the body odour of others, but to what extent we can access this information about ourselves remains a subject for future research. In **Chapter 5,** I introduce other modalities that could work in concert with olfaction: they can either deliver the same information about the conspecific (the 'backup signals' hypothesis) or, alternatively, it is possible that each modality carries different information about the perceived individual (the 'multiple messages' hypothesis). Then I discuss the gap in multimodal research, noting that we know little about relations between olfaction and other modalities and how they may interact during perception. In **Chapter 7**, I review the evidence regarding the behavioural immune system, its components which lead to successful pathogen avoidance, and discuss how this system may be associated with the obsessive behavioural and obsessive–compulsive disorder. The functioning of the behavioural immune system during a naturally occurring coronavirus pandemic is demonstrated in an empirical study fully described in **Chapter 8**. The study presented in **Chapter 9** illustrates an alteration in the body odour as well as facial and vocal attractiveness after vaccination against viral and bacterial diseases. In **Chapter 10**, I review olfaction-mediated pathogen avoidance while paying special

attention to nonhuman mammals alongside humans and noting some future directions of this research area. **Chapter 11** demonstrates that there is no association between immunoreactivity induced by vaccination and facial attractiveness. The following **Chapter 12** introduces the second part of results previously presented in Chapter 11 and discusses associations between the functioning of the immune system (i) after stimulation and ii) without stimulation and the quality of the body odour. In **Chapter 13**, I present qualitative data regarding how partner's body odour influences women during intimate and sexual situations, including the negative impact of the body odour of partner who is ill. The penultimate **Chapter 14** discusses the frequency, incidence, and possible functions of olfactory self-inspection in humans. The last **Chapter 15** presents a meta-analysis of how the ratings of body odour attractiveness are (not) linked to the ratings of facial and vocal attractiveness.

1. WHY IS BEHAVIOURAL DEFENCE IMPORTANT?

The physiological immune system (PIS), especially its adaptive part, is constantly challenged by pathogens, which coevolve to evade our defence responses (Tenthorey et al., 2022). The hosts' PIS may react to pathogens by resistance, tolerance, or by avoiding them before coming into contact. But if a pathogen already entered the organism, the PIS needs to take several important 'decisions'. The first pertains to whether the PIS responds to that specific pathogen or tolerates it within the body. The second deals with the kind of response that should be triggered to cope with that particular pathogen.

When a pathogen enters the body, it immediately triggers the innate immune response, which is not specific and acts within minutes of pathogen exposure. The innate immunity relies on a limited number of receptors but seems to focus on conserved microbial components shared by large groups of pathogens. This leads to a general protective immune response. An important part of this innate immunity is responsiveness of the skin and epithelial cells of the respiratory or gastrointestinal tracts (Turvey and Broide, 2010). The survival of most organisms on the planet depends on this part of the immune system.

Vertebrates 'improved' on their immune system by adding an adaptive part, which works alongside the innate immune system. This evolutionarily younger part of the PIS relies mainly on dendritic cells (DC) and other antigen-presenting cells, which function as sensors that inform the system about the presence of a dangerous pathogen in the body (Banchereau and Steinman, 1998). Once these cells detect the pathogen, they leave the site and migrate towards immature T cells in secondary lymphoid organs to present the information. This triggers an adaptive and highly specific immune response. In reaction to the infection, lymphocytes undergo a clonal expansion, which is crucial for an efficient immune response. But it takes a few days to produce a sufficient numbers of clones and, in the meantime, the pathogen could damage the tissues and the body as a whole. Addressing the issue, the adaptive immune system is capable of maintaining an immune memory within lymphocytes; thanks to this, it can later trigger a faster response when exposed to the same antigen (Pulendran, Palucka, and Banchereau, 2001).

Pathogen defence is achieved by orchestrating the response of the innate and adaptive immune systems with help the of proinflammatory mediators to promote the defence and restoration of damaged tissues. Then there come the anti-inflammatory mediators responsible for controlling the inflammation and restoring immune homeostasis. If this balance is disrupted and excessive

immune response is active for a prolonged period time, this can potentially lead to a septic shock (Vincent, 2002).

Activation of the PIS thus leads to an effective defence against various pathogens and toxic substances but can also pose a risk to the body. Inadequate response can harm the tissues and lead to autoimmune diseases or the abovementioned septic shock. Moreover, many symptoms of disease (such as fever or fatigue) are caused by the immune system response itself. The PIS should therefore deal with pathogens in a way that does not lead to a waste of energy and resources while avoiding the initiation of (deleterious) autoimmune responses. A single mistake in the autoimmune response can have lethal consequences but any immune response is also energetically demanding. An effective immune response involves an increase in secretory and cellular proliferative activities which require a lot of energy. The most essential source of this energy is glucose, but lipids such as fatty acids or oligoelements, such as zinc or iron, are no less important (Wolowczuk et al., 2008). This implies that it is crucial for the physiological immune response to be activated only when a pathogen enters the body and resistance is the only option left.

Then there is also another way of coping with pathogen threat, one that avoids the activation of a fully orchestrated immune response altogether: it operates by decreasing the likelihood of pathogen transmission and is known as the behavioural immune system (BIS). It consists of a set of physiological mechanisms leading to behavioural avoidance of potential sources of contagion (Schaller and Park, 2011). Naturally, most pathogens are too small to be perceived by mere eye or detected by other senses. The whole system therefore depends on cues which are 'left behind' by the pathogen activity. Conspecifics who show signs of illness, someone else's faeces, foul smell of rotten meat – all of these can serve as cues. Detection of such cues triggers an affective component of the system, with disgust as the leading emotion (Oaten, Stevenson, and Case, 2009). Moreover, a cognitive component, which allows us to be aware of the degree of threat and to discern which stimuli are potential pathogen sources by social learning (i.e., without direct experience) also plays an important role in the BIS. This component also allows us to consider the risk when avoidance is not possible. Importantly, behavioural avoidance is facilitated by cooperation between all these three components.

Although there can be a straight line between the perception of threatening stimuli and the disgust response, the system is much more sensitive and even things which pose no risk but resemble the threatening stimuli, such as vitiligo (a skin condition described as patchy loss of

skin pigmentation, which is not contagious) (Barve , 2021), may lead to a strong BIS response. The functioning of the system can be explained by the error management theory (EMT), which proposes that a system should favour less energetically costly errors (Johnson et al. 2013). This indicates that the BIS operates based on the 'smoke detector principle' (Nesse, 2005), in other words, that it is skewed in favour of false-positive errors because their impact is less costly and this strategy limits the occurrence of false-negative errors which could be fatal. This sensitivity may lead to social consequences, for example, when people are avoiding another person with a noncontagious eczema or with a limb missing due to a car accident (Schaller, 2011). Even obese people or the elderly are victims of prejudicial responses which may result from a false overgeneralisation of the BIS (Duncan and Schaller, 2009).

Apart from the behavioural avoidance outlined above, it seems that BIS is also functionally linked to the PIS. According to the compensatory prophylaxis hypothesis, the BIS may compensate for compromised functioning of the PIS, for instance during ageing or in the early stages of pregnancy (Fessler et al., 2005). According to this hypothesis, disgust sensitivity should thus be elevated when the PIS is functionally compromised and, in fact, we found that older respondents reported overall higher pathogen and moral disgust during the Covid pandemic (Schwambergová et al., 2023). Furthermore, elevation in disgust sensitivity was negatively associated with the levels of a wide range of cytokines in early pregnancy (Kaňková et al., 2023).

Furthermore, the BIS may help prepare the organism for possible contagion and mobilise the PIS in case pathogen contagion has already taken place. This finds support in findings such as that stimuli showing diseased-looking people trigger the production of significantly more pro-inflammatory interleukin 6 (IL-6) than photographs depicting guns (Schaller et al., 2010). Even disgusting odours triggers an increase in the levels of tumour necrosis factor alfa (TNF- α) more than neutral odours do (Juran et al., 2023). Similarly, social partners of rats injected with lipopolysaccharide (LPS) displayed increased levels of interleukin-1β (IL-1β), IL-6, and TNF-α (Hamasato et al. 2017). This indicates that the BIS and PIS do not work independently: they modulate each other according to the current situation. Exposure to a potential pathogen source leads to an increase in pro-inflammatory cytokines which are active during inflammation. On the other hand, as mentioned above, the BIS is sensitive to any cues, even 'fake' ones, so the innate immune response should, in theory, be readied in non-threatening situations as well. To the best of our knowledge, though, no study as yet investigated this question. We thus do not know whether the PIS response is general, that is, triggered by whenever the organism detects

a possible source of contagion, or/and is affected by other factors, for example self-assessed vulnerability to diseases. Future studies should address this issue to better understand the relationship between the PIS and the BIS. To read more about the functioning of the BIS, its components and possible social implications, see Chapter 7.

2. HOW CAN WE ASSESS THE BIS?

To test the BIS, researchers use various tools which have different advantages and disadvantages. The BIS is usually tested under laboratory conditions or via online questionnaires, with focus mainly on the perceptual and affective components of the system. The tools most frequently used to study the BIS and disgust are questionnaires, such as the Disgust scale (DS) (Haidt, McCauley and Rozin, 1994), Disgust Scale – revised (DS-R) (Olatunji et al., 2007), the Three-Domain Disgust Scale (TDDS) (Tybur et al., 2009), Perceived Vulnerability to Disease (PVD) (Duncan, Schaller and Park, 2009), or the Body Odor Disgust Scale (BODS) (Liuzza et al., 2017). Respondents depend on their imagination to process situations presented in the text, because the abovementioned questionnaires mostly inquire how disgusted one would feel in certain situation, when confronted with certain stimuli, or whether one agrees with certain presented statements. They cover a number of disgust domains, which are based on disgust elicitors, such as food, body products, or death. The questionnaires nowadays seem to be chosen relatively freely and they are used as if they were interchangeable. But that need not be the case: each of the abovementioned questionnaires may cover different parts or domains of disgust. For example, the DS-R and TDDS are used to measure overall disgust sensitivity although the TDDS differentiates and is accordingly divided in pathogen, moral, and sexual domains (Tybur et al., 2009), while the DS-R measures the core, contamination, and animal-reminder domains (Olatunji et al., 2007). They are both frequently used, but from the DS-R the sexual and moral items were removed to produce a more homogenic disgust measure. As a result, though, it does not measure overall disgust but rather the tendency to experience one type of disgust (Tybur and Karinen, 2018). The TDDS pathogen domain corresponds to all DS-R domains (Olatunji et al., 2012). Because disgust sensitivity may be influenced by self-perceived vulnerability to disease, the PVD questionnaire could be a useful tool when we want to measure the motivation of pathogen avoidance. The BODS is nowadays the only widely used questionnaire which assesses the disgust elicited by human body odour. To the best of my knowledge, though, no questionnaire measures overall odour-mediated disgust.

One may argue that the BIS evolved to respond to perceptual cues of infection but not to indirect stimuli; on the other hand, one could argue that at least affective states are quite easily induced by symbolic communication in the written form (Calvo and Kim, 2013). Moreover, questionnaires are easily distributed to respondents and can be completed online. Still, self-report tools always have some inherent limitations. On top of that, while some of these tools may be good for measuring interindividual differences they need not be sensitive enough to record intraindividual differences during repeated measurements (Fleischman and Fessler, 2018; Schwambergová et al., 2023).

Another approach to measuring the BIS relies on the presentation of stimuli pairs which do or do not depict a potential disease threat and subsequent rating for disgust. These sets of stimuli are mainly visual. The older set by Curtis et al. (2004) does not have a optimally balanced stimuli pairs and misses some of the key sources of potential contagion, e.g., faeces or decaying bodies. The newer set, called the Culpepper Disgust Image Set (C-DIS), has been carefully assembled to cover all potential key sources of pathogens and consists of twenty pairs of stimuli (Culpepper et al., 2018). Such visual sets are considered to be a more sensitive measure of variation in pathogen disgust than the textual approaches described above, but analogical universal sets of acoustic, olfactory, or tactile stimuli are currently not available.

Behavioural avoidance tasks (BAT) are standardised behavioural tests which are supposed to evaluate avoidance induced by a disgusting stimulus. For example, researchers record whether participants are willing to hold and taste a cookie or handle a hair comb presented on the ground (Deacon and Olatunji, 2007). In another study, participants were asked to touch tissues that had been used by someone with a cold (Fan and Olatunji, 2013) or to touch a colonoscopy bag (Reynolds et al., 2014). Willingness to handle the abovementioned things was negatively predicted by pathogen disgust levels. Moreover, pathogen disgust predicted avoidance of public toilets, sinks, or trash cans (Olatunji et al., 2012).

Aside from these tools and methods, there is also the experimental approach which allows researchers to control the context and simulate situations that would be difficult to observe spontaneously. It also allows for monitoring the behavioural component, i.e., the avoidant or prophylactic behaviour (such as increased hygienic behaviour), of the BIS. One such study showed that a group of participants experimentally exposed to faecal odour reported a higher likelihood of buying and using condoms, because condoms significantly reduce the threat of infection with sexually transmitted diseases (Tybur et al., 2011). Exposure to putrescin, a

chemical compound produced in decaying bodies, elicited distancing and increased the speed with which participants walked away from the odour location (Wisman and Shrira, 2015). Recently, even virtual reality is starting to be used in disgust research. For instance, Ammann and colleagues (2020) measured people's willingness to eat a piece of chocolate after exposure to a virtual disgust cue (dog faeces). Participants exposed to this disgust cue were more likely to refuse consumption than participants in a control condition.

All of the abovementioned tools and methods are used in research of the BIS, disgust, and pathogen avoidance under controlled conditions. But starting with the end of 2019 we had an opportunity to investigate a 'naturally occurring experiment'. Coronavirus SARS-COV-2 had appeared and for several years that followed, it influenced the lives of all people on Earth. This situation was a challenge but also an opportunity for BIS researchers who started monitoring natural changes in people's behaviour. Xenophobia and discriminatory attitudes towards mainly Asians appeared in the early stages of the pandemic – this was associated with concerns about the virus (Reny and Barreto, 2022). Hygiene and sanitation were not just recommended by the World Health Organization but mainly hand hygiene had actually increased during the time of pandemic (Głąbska, Skolmowska, and Guzek, 2020). During lockdowns but also at other times during the pandemic, social distancing was not only recommended but also maintained in an effort to reduce transmission intensity (Kissler et al., 2020). Researchers found that during the pandemic, respondents reported higher disgust sensitivity to disgust-evoking pictures (Milkowska et al., 2021) and scored higher on the DS-R questionnaire (Stevenson, Saluja and Case, 2021) than before the pandemic. In another study, Covid-19 concerns as well as prophylactic behaviours, such as social distancing or mask wearing, were positively associated with the germ aversion subscale of the PVD and the pathogen domain of the TDDS (Shook et al., 2020). This evidence strongly suggests an activation of BIS due to a naturally occurring pandemic. Nevertheless, when we compared one group of participants in the early stages of the pandemic in early 2020 and later, after the restrictions were lifted in summer 2021, we found no difference in pathogen disgust; see Chapter 8. This may indicate that while the objective threat was quite high (and people were constantly reminded of it by the media and authorities), most people were not during the lockdown exposed to direct pathogen cues (in the form of, for instance, coughing people).

2.1. Challenges for the BIS and disgust research

This brings up another topic concerning the functionality of the BIS. As noted above, this system should be highly sensitive and overgeneralise infection cues. On the other hand, a considerable percentage of people who got infected with Covid-19 remained asymptomatic, thus failing to present cues from which the virus could be detected. Some researchers even argue that Covid-19, like many other respiratory pandemic diseases, did not trigger the BIS response because the cues it presented were too weak and asymptomatic transmission frequent (Ackerman, Tybur, and Blackwell, 2021). In humans, the BIS evolved in small groups and mainly in response to parasites, which do not have a pandemic potential, which is why pandemics with nonsufficient cues may be beyond its detection ability. Further studies should focus on the functioning of BIS during seasonal waves of respiratory diseases in a longitudinal manner to see whether disgust sensitivity changes or fluctuates.

Still, although a disease may present no sensory cues that could be detected, the cognitive component of BIS may compensate for it and some aspects of the system, such as social distancing, mask wearing, or heightened hygiene behaviour, may still take place. Future studies should investigate the various components of the BIS and their interactions. For example, would one observe the same behavioural outcome if there were two experimental places, one where infection cues are present and another, where there would be only notices warning of a potentially threatening item somewhere?

Aside from that, one may ask how salient the visual, acoustic, olfactory, or tactile cues need to be to trigger the BIS. Visual contact with a potential source of contagion is different than direct contact by touching: the latter should lead to a more urgent activation of behavioural response. To the best of my knowledge, however, no study as yet investigated this issue.

3. THE ROLE OF OLFACTION IN BEHAVIOUR DEFENCE

The olfactory modality has many benefits in terms of avoidance behaviour. It has a number of unique features: odours can not only travel long distances but also stay in one place as a marker which can alert conspecifics that the place had been visited by a potentially infected individual and should best be avoided. Some odour molecules remain stable for a number of hours or even days (depending on their volatility and degradability). Finally, in situations where we cannot effectively use our vision, for instance at night, or hearing, for example in a noisy environment, olfaction remains fully functional (Wyatt, 2003). An indisputable benefit of using olfactory

cues is that one does not need to be in direct contact with the potentially infected individual because olfactory cues can usually be detected at greater distances.

The sense of smell has been traditionally used to detect potential contamination of food (e.g., by sniffing milk or meat). Edibility can also be indicated by visual cues, such as mould, or by gustatory cues, such as bitter taste, which is often linked to toxins. But odours, such as the smell of decaying food, are the most prominent cues that can be used without getting in direct contact with the food. Foul smell is the by-product of activity of various bacteria and fungi responsible for food rotting. Consumption of such food could have serious consequences ('food poisoning'). Aside from that, olfaction also plays a significant role in the chemical communication between people and assists us in making sure that the environment we are in is safe. Most people would avoid or try to quickly leave a place where they can detect the smell of decomposing bodies or faeces. Disease-related changes in body odour were reported already at the time of Hippocrates; it was known that various diseases are characterised by a distinctive smell (Havlíček, Fialová and Roberts, 2017). Infectious diseases and inflammation tend to affect the odour of breath, sweat, vaginal fluid, urine, as well as faeces.

3.1. Does olfaction-mediated behavioural defence play a role in mate choice?

It should be noted that most studies on behavioural immune defence focus on preferences and attractiveness ratings, which are important in mate choice, but organisms need to defend themselves during other contacts with conspecifics with a similar effectiveness as well.

Preferences for a potential partner can be divided in two categories. First, there are the direct benefits, which include immediate qualities such as ability to provide resources, protection, or territory. Current health status also falls into this category, because – in addition to posing a lower risk of disease transmission – a healthier partner can provide better parental care (Kirkpatrick and Ryan, 1991). Secondly, there are indirect benefits pertaining to the individual's quality: selection of a partner with 'good genes' can contribute to getting better mates or pathogen resistance in the offspring via inheritance (Hamilton and Zuk, 1982, Gangestad, 1993).

It is therefore important to distinguish between studies that focus on the current health status and those which investigate overall immune qualities of individuals, because the two are not interchangeable. As noted above, contact with ill individuals can have consequences for any conspecifics, which is why preference for social interaction with currently healthy individuals

should be of importance for everyone (and not only in the context of mate choice). On the other hand, individual and genetic quality is more important in mate choice because it has an impact especially on the offspring.

3.1.1. The importance of current health in a partner

Evidence supporting olfaction-mediated defence mechanism in humans is limited, but existing studies all show the same trend. It has been shown that female raters find axillary body odour collected from individuals infected with gonococcus (*Neisseria gonorrhoeae*) less pleasant and more putrid than samples collected from healthy or recovered donors (Moshkin et al. 2012). Another study collected samples from donors who were currently sick with a naturally occurring acute respiratory infection; these odours were rated by both sexes as less pleasant, less healthy, and more intense and disgusting than samples collected from the same donors when they were in full health (Sarolidou et al. 2020b). Humans are capable of detecting activated immune response even in absence of any pathogen present in the body. Within a few hours after an activation of the immune system by a lipopolysaccharide (LPS) injection, body odour samples collected from donors were perceived as less pleasant, less healthy, and more intense than samples from the same donors after a placebo injection (Olsson et al. 2014). Interestingly, two weeks after vaccination (used as an activator of immune system response) collected samples of body odour were rated as more attractive, healthier, and less intense than samples collected before vaccination (Schwambergová et al. submitted, see Chapter 9). This shift in attractiveness and pleasantness should be tested in future studies, because even the body odour of patients who had recovered from gonococcus were more likely to be rated as floral (Moshkin et al. 2012).

The decrease in preference for urine odours of infected mice or rats is thoroughly documented, but it seems that any subsequent avoidant behaviour may be influenced by numerous other factors. Preference and investigation tests conducted mostly in Y-mazes or in special chambers show a rather straightforward preference for healthy – rather than parasite-infested – individuals. Moreover, the level of analgesia (reduced pain sensitivity) assessed on a hot plate (50–60°C) where vigorous foot flutter or licking is recorded and thermal latencies observed, likewise shows an increasing tendency after exposure to the urine odour of infected individuals. Analgesia helps the organism prepare for a fight or flight reaction and, as it turns out, longer exposure to urine of infected individuals leads to higher levels of analgesia induced by opioid compounds, which also play role in the adaptive preparation of the PIS (Stefano et al. 1996).

This mechanism is in some respects parallel to the observed increase in pro-inflammatory cytokines levels in humans after presentation of a potentially risky stimulus (Phalane et al., 2017).

Nevertheless, pathogen avoidance can be modulated by various factors, such as the pathogenicity, familiarity, or dominance of the pathogen. For example, in arena setting that allowed for a direct contact female mice initially favoured healthy males but ultimately the reproductive success of healthy and infected males did not differ (Zala et al. 2015). As noted above, what seems to play an important role in mate choice is probably the pathogen's pathogenicity and if it is low, reproductive success may become more important. Another important factor modulating odour preference might be the mating system: polygynous or promiscuous species are more vulnerable to pathogen transmission (Klein et al. 1999), which is why one can expect them to be more sensitive to various markers of the health status of potential partners. It also seems that prior experience increases the level of caution regarding the health status of a potential mate (Edwards and Barnard 1987). More on olfaction-mediated pathogen avoidance in mammals can be found in Chapter 10.

As indicated above, numerous factors influence both odour preference and actual reproductive success. Our efforts to understand them better may be affected by the fact that most studies on this subject were conducted on rodents, with only a handful involving other non-human mammals, such as primates or banded mongooses. We have a relatively good general understanding of the topic, but majority of available evidence comes from laboratory conditions and is based on model organisms. We need to do more research on wild populations, because odour preference based on laboratory tests may differ from the actual mate choice. Research should also include a wider variety of mammalian species. Such extension of research would allow us to draw more general conclusions.

3.1.2. The importance of partner's immune quality

When choosing a partner, cues to a good immune quality should be among the most important ones. One of the aspect of functionality of the immune system is immunoreactivity, that is, the magnitude of organism's response to antigens. Some clinical studies had shown that increased levels of antibodies after vaccination might indicate a higher effectiveness of the immune system (Burns and Gallagher, 2010). In fact, some studies on facial attractiveness reported that the faces of males with higher levels of antibodies after vaccination against hepatitis B were rated as more attractive (Rantala et al., 2012), but no analogous pattern was observed in females

(Rantala et al., 2013b). Another study reported a nonsignificant negative association between facial attractiveness, vocal attributes, body height, and immune system reactivity in men (Skrinda et al., 2014). Immunoreactivity might be indicated by the be levels of cytokines, important signal peptides which play a crucial role in the immune response to infections and in inflammation. In fact, a study by Phalane et al. (2017) showed a positive association between perceived facial attractiveness, health, and elevated levels of cytokines upon activation by an injection of LPS.

In our study, we found no association between the level of increase in antibodies against hepatitis A and meningococcus after vaccination and perceived facial attractiveness and healthiness. We anticipated that participants with a stronger immune reaction (measured by increased levels of specific antibodies) would be perceived as more attractive and healthier. This subject is discussed in more detail in Chapter 11. Empirical evidence regarding an association between immunocompetence and facial attractiveness is thus equivocal (Jones et al., 2021). Importantly, to focus solely on antibody levels seems to be excessively simplifying since antibody levels provide only limited information about the functioning of the immune system.

Odour can likewise function as a cue to immune system functioning and immunoreactivity. For example, after activation by LPS, odour samples were perceived as more aversive, and the effect was mediated by pro-inflammatory cytokines IL-6 and TNF- α (Olsson et al., 2014). It is possible, though, that there is a dissonance between the attractiveness of the body odour and the face. For instance, body odour samples tend to be rated as less pleasant after activation of the immune system with LPS injection, which leads to increased levels of pro-inflammatory cytokines, than body odour samples after a saline injection which does not change cytokine levels. On the other hand, the faces of males who had been injected with LPS were rated as more attractive and healthier in those individuals whose cytokine levels increased compared to those whose cytokine levels increased less (Phalane et al. 2017).

To the best of my knowledge, our study presented in Chapter 12 is so far the only attempt to systematically investigate the relationship between body odour attributes and the functioning of the immune system. We found no association between the rate of increase in antibodies against hepatitis A and meningococcus after vaccination and body odour attractiveness, intensity, or healthiness. Similarly, we found that body odour attributes were not associated with innate or adaptive immune system markers without stimulation in healthy individuals. It

should be noted, though, that higher immune responsiveness does not necessarily imply higher disease resistance, because hypersensitivity of the PIS can have a negative impact on individual viability and fitness. Moreover, various non-elevated immunological parameters need not be easily perceptible by olfaction.

To sum up, there is an emerging body of evidence which shows that odours can serve as a sufficient cue to current health status but evidence concerning the association with at least genetic quality is limited.

4. IS THE BIS ASSOCIATED WITH OLFACTORY SELF-INSPECTION?

The largest part of the thesis focuses on how we interpret cues from the body odour of others to arrive at assessments regarding their health or overall quality. This leaves one important question: Can we inspect our own body odour to acquire information about our own current health?

We may observe this behaviour in nonhuman mammals but there is not much direct evidence. For example, we assume that olfactory self-inspection may appear in form of inspection of own urine marks in grey wolves (Cazzolla Gatti et al., 2020) or self-inspection of genitals in female lemurs after mating (Palagi, Telara, and Tarli, 2003). Still, it seems safe to assume that for example rodents, who heavily rely on olfactory communication, sniff themselves during self-grooming activities. In this way, they may be able to control how much information they provide to other conspecifics (Scauzillo and Ferkin, 2020). Another kind of a possible olfactory self-inspection has been observed in apes and humans. Face-touching at a rate of about once a minute has been observed in gorillas, chimpanzees, and orangutans (Dimond and Harries, 1984), while humans touch their faces about 10 to 23 times an hour (Nicas and Best, 2008; Elder et al., 2014; Kwok et al., 2015).

Several previous studies have shown that olfactory self-inspection is a rather common behaviour in humans. In humans, self-sniffing (used as synonym for olfactory self-inspection) of certain body parts (private areas) cannot be observed directly for ethical reasons, which is why researchers must rely on self-reports. This may, however, be somewhat problematic because self-sniffing is probably at least in part unconscious. The first study on this subject indicated that over 90% of respondents reported sniffing their hands or armpits and 73.9% of men and 55.7% of women reported sniffing own hand after touching their crotch (Perl et al., 2020). To investigate this behaviour more deeply, Li et al., (2022a) developed the Body Odor

Sniffing Questionnaire, which covers also other body parts as well worn clothes and bedding. Their results showed that Chinese and US respondents differ in which body parts they frequently smell, with Chinese respondents smelling more their own 'public' odours (armpits, T-shirt etc.) than US respondents, who sniffed the 'private' odours more frequently. Furthermore, women tended to sniff themselves more than men do (Li et al., 2022a). Their second study showed a similar pattern but also found that people who engage more in self-sniffing report a stronger sexual desire (Li et al., 2022b). Still, all in all, little is known about the function of this behaviour.

As noted above, body odour conveys information about for instance the health status (Olsson et al., 2014), diet (Zuniga et al., 2017; Fialová et al., 2019), emotional states (de Groot, Semin, and Smeets, 2017), or hygiene. If humans can perceive changes in the body odour of their romantic partners which are due to an illness or changes in the diet (see Chapter 13), if they can in detail report how it affects them, it seems they should be able to detect alteration in their own body odour as well.

In our study, we investigated self-sniffing, in particular the frequency of this behaviour with respect to different parts of the body or worn clothing, but also considered the evolutionary functions of olfactory self-inspection, namely monitoring of own health and hygiene status. On top of that, we also wanted to see whether the frequency of self-sniffing frequency is modulated by disgust sensitivity.

We have identified three main axes of self-sniffing: social acceptability self-inspection (sniffing of body parts such as the armpits, breath, or own worn clothes), intimate self-inspection (sniffing of body parts such as genitals, the anal area, and the navel), and cosmetic self-inspection (sniffing of body parts such as the hands, hair, or neck via hand). We found that each dimension of olfactory self-inspection seems to have a different function and may carry specific information about own status. Our results showed a positive association between self-reported health issues and social acceptability self-inspection score, meaning that people who more frequently sniff their armpits, breath, or worn clothes also report more frequent health issues. It seems therefore that although the BIS primarily helps detect possible pathogen threats in the environment or in conspecifics, its other function could be the detection of own impaired health. This would be beneficial because the ill individual could withdraw from social interactions and thus decrease the likelihood of pathogen transmission to other individuals, including relatives.

Even smelling of own private areas, mainly in women, may be a way of checking own health. Vaginal infections can change vaginal odour, which serves as one of the first indicators of these health problems (Tempera, 2005). We found no association between intimate self-inspection and self-reported health issues, but then again when we asked about common short-term and long-term health problems (e.g., headaches, cough, bellyaches) vaginal infections were not part of the list. Furthermore, intimate self-inspection was negatively associated with hygiene habits, which may indicate that respondents who reported lower hygienic habits might compensate for it by increased intimate self-inspection to control their body odour.

The last dimension, cosmetic self-inspection, was associated neither with self-reported health issues nor with hygiene habits. It seems therefore that this dimension is not connected to the BIS but may serve rather as an act of self-soothing, reassurance, or be used as a way of enjoying used fragrance. For more detailed results and discussion, see Chapter 14.

Future studies should investigate the contexts in which people sniff certain body parts and complement the self-report questionnaires with direct observations of self-sniffing of at least of some body parts which are not necessarily tied to private settings.

5. ASSOCIATIONS BETWEEN OLFACTION AND OTHER MODALITIES

In many different taxa, individuals obtain information about the outside world by various modalities. This includes detection of potential source of pathogens in the environment but also assessment when in contact with a conspecific. In many cases, to use only a single modality would be ineffective: perception by only one modality could fail to detect some important information. In general, the perception of cues/signals has been considered in the context of two main hypotheses. The first is the 'multiple messages' hypothesis, which proposes that each cue or signal provides unique, independent, and nonredundant information about an individual's condition and quality (Möller and Pomiankowski, 1993). It implies that cues from different modalities complement each other, which leads to a more accurate assessment of an individual's quality that perception by any single cue could. According to the 'backup signals' hypothesis, on the other hand, cues or signals provide similar, redundant, and overlapping information about the individual (Möller and Pomiankowski; 1993, Grammer et al., 2001). Assessment of such information from different modalities should therefore reduce the likelihood of errors. To sum up, both hypotheses assume the goal of accurate assessment of an individual's quality but suggest different ways in which this is achieved.

The results of our study (in Chapter 9) indicate that various studied modalities (body odour samples, facial photographs, and vocal recordings) carry different information. After immunoactivation by vaccination, body odour samples were rated as more attractive and less intense while post-vaccination facial photographs were rated as less attractive and less healthy, while vocal attractiveness before and after vaccination did not differ at all. Still, our results thus seem to support the 'multiple messages' hypothesis: every modality seems to carry different information about the individual's post-vaccination condition, although the abovementioned associations were mostly weak. Future studies should collect body odour samples, facial photographs, and voice recording at several time points to detect possible shifts in attractiveness and healthiness assessments from different modalities.

Subsequently, we analysed various datasets focused on relationships between human body odour and facial attractiveness and between human body odour and vocal characteristics. We found that body odour and facial attractiveness as well as body odour and vocal attractiveness are positively associated, but the effects are very small. Based on these findings, it would seem that body odour provides unique and nonredundant information about the individual, which supports the 'multiple messages' hypothesis; for more details see Chapter 15. These results are in line with a recent study conducted using the speed-dating paradigm, where only weak associations between body odour, facial, and vocal attractiveness were found. The ratings of visual attractiveness showed a strong positive correlation with desirability to meet the partner again, while the effects of olfactory and auditory attractiveness were negligible (Roth et al., 2021). It is even possible that cues from different modalities play a different role at different stages of mate choice.

5.1. Multimodal perception

To the best of my knowledge, there are currently no studies conducted on rodents or other nonhuman mammals investigating multimodal perception of infection cues in conspecifics. Still, there are some studies that had been conducted on humans which could provide relevant evidence. When participants were asked to rate the attractiveness of facial photographs after LPS treatment, they were perceived as less likable, less healthy, and less attractive if presented simultaneously with body odour collected after LPS treatment (Regenbogen et al., 2017). Similarly, participants rated as less likable facial photographs and body odour collected after (as opposed to before) LPS treatment (Sarolidou et al., 2020a). Further studies on multimodal

perception need to be conducted to shed light to how the different modalities interact when presented simultaneously in the context of assessment of current health status.

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PART II.

Chapter 7

ROLE BEHAVIORÁLNÍHO IMUNITNÍHO SYSTÉMU V OBRANĚ PROTI INFEKCÍM

ROLE BEHAVIORÁLNÍHO IMUNITNÍHO SYSTÉMU V OBRANĚ PROTI INFEKCÍM

souborný článek

Dagmar Schwambergová
Žaneta Slámová
Jitka Třebická Fialová
Jan Havlíček

Národní ústav duševního zdraví, Klecany

Kontaktní adresa:
Mgr. Dagmar Schwambergová
Národní ústav duševního zdraví
Topolová 748
250 67 Klecany
e-mail:
Dagmar.Schwambergova@nudz.cz

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SOUHRN

Schwambergová D, Slámová Ž, Třebická Fialová J, Havlíček J. Role behaviorálního imunitního systému v obraně proti infekcím

Behaviorální imunitní systém představuje vedle tělesného imunitního systému další obranný nástroj organismu. Jeho hlavní funkcí je detekce a vyhnutí se potenciálně ohrožujícím podnětům, jež mohou vést k přenosu patogenů. Včasná vyhýbavá reakce organismu významně snižuje energii, jež by jinak byla vydána na metabolicky náročné reakce tělesného imunitního systému po infekci patogenem. Cílem tohoto souborného článku je představit jednotlivé složky behaviorálního imunitního systému, jenž v první řadě zahrnuje percepci ohrožujících podnětů na základě jednotlivých smyslových modalit (vizuální, akustická, čichová, taktilní a jejich integrace). Dále se jedná o afektivní složku, a to zejména emoci znechucení, kterou zde dále dělíme na patogenní, sexuální a morální. Další části zahrnují kognitivní složku, která umožňuje uvědomovat si a hodnotit míru nebezpečnosti podnětu, a exekutivní složku včetně vyhýbavého chování.

Součástí práce je také krátké představení základních metod měření behaviorálního imunitního systému pomocí dotazníků, vizuálních stimulů či měření fyziologických reakcí. Fungování behaviorálního imunitního systému je možné vysvětlit pomocí „teorie zvládání chyb" (error management theory), podle níž systém funguje ve prospěch energeticky méně náročných chyb. Kvůli vysoké citlivosti behaviorálního imunitního

SUMMARY

Schwambergová D, Slámová Ž, Třebická Fialová J, Havlíček J. The role of behavioural immune system in infection avoidance

The behavioural immune system is, besides the body immune system, another defence mechanism of the organism. Its primary function is to detect and facilitate avoidance of potentially harmful stimuli that can lead to pathogen transmission. The prompt avoidant reaction significantly reduces high energetic demands that would be spent by the body immune system after the actual pathogen infection. The main aim of this review is to introduce individual components of the behavioural immune system that, first, include the perception of the threatening stimuli based on the individual sensory modalities (visual, acoustic, tactile and their integration). Next is the affective component, which primarily involves the emotion of disgust. Here, we differentiate three domains: pathogen disgust, sexual disgust and moral disgust. Other components include cognitive processes that enable awareness and evaluation of the extent of the threat and executive component, including avoidant behaviour.

The paper also shortly introduces the major testing methods of the behavioural immune system, such as questionnaires, visual stimuli and measurement of the physiological responses. The functioning of the behavioural immune system is best explained by error management theory which predicts that less costly errors are favoured. The high sensitivity of the behavioural immune system thus decreases

systému se snižuje množství falešně negativních chyb, ale naopak se zvyšuje náchylnost vůči falešně pozitivním chybám. V důsledku generalizace pak tyto reakce mohou mít zásadní sociální implikace, jako je vliv na společenskost, ageismus, xenofobii a konformitu vůči normám. V neposlední řadě poukážeme na možnou souvislost mezi behaviorálním imunitním systémem a vznikem některých psychiatrických poruch, jako je obsedantně-kompulzivní porucha. Systém je funkčně flexibilní dle možné zranitelnosti jedince a aktuální situace, proto se stručně věnujeme i jevům souvisejícím s aktivací behaviorálního imunitního systému objevujícím se v současné situaci pandemie COVID-19.

Klíčová slova: čich, imunita, konformita, obsedantně-kompulzivní porucha, patogeny, percepce, sexualita, xenofobie, znechucení.

the amount of false-negative errors and as a result, increases the susceptibility to false-positive errors. Due to overgeneralization, these reactions may have significant implications for social life, with an influence on social gregariousness, ageism, xenophobia and conformity to social norms. Finally, we point to a possible association between the behavioural immune system and the development of some psychiatric disorders such as obsessive-compulsive disorder. The system is functionally flexible according to potential disease susceptibility and current situation. Therefore, we briefly discuss the issues related to activation of the behavioural immune system in the current COVID-19 pandemic.

Key words: conformity, disgust, immunity, obsessive-compulsive disorder, olfaction, pathogens, perception, sexuality, xenophobia.

ÚVOD

Nutnost obrany proti patogenům představuje jeden z nejvýznamnějších „motorů“ evoluce. Platí to především u sociálně žijících organismů s vyšší populační hustotou, kde je riziko přenosu patogenů nejvyšší. Člověk v tomto ohledu není výjimkou, protože infekční onemocnění byla po většinu lidské evoluce zodpovědná za větší část úmrtí než všechny ostatní příčiny dohromady. Hlavním nástrojem obrany organismu proti patogenům je tělesný imunitní systém (TIS) a na pochopení jeho fungování se donedávna soustředila většina evolučně zaměřených výzkumů. Nicméně TIS není jediným způsobem obrany organismu. V poslední době se do popředí zájmu dostává také behaviorální imunitní systém (BIS), jehož úkolem je směrovat chování jedinců tak, aby se minimalizoval kontakt s potenciálními zdroji infekce. Cílem tohoto souborného článku je představit BIS a jeho percepční, afektivní i behaviorální složky. Dále ukážeme, jaké mohou mít procesy fungování BIS dopady na sociální chování (např. výběr partnera, xenofobie či konformita) a vznik některých psychiatrických onemocnění (zejména obsedantně-kompulzivní poruchy).

JAKÝ JE VÝZNAM BEHAVIORÁLNÍHO IMUNITNÍHO SYSTÉMU?

TIS savců tvoří celá řada vzájemně propojených procesů, které slouží k rozeznávání struktur tělu vlastních a cizích (škodlivých i neškodných), na něž následně adekvátně reaguje. TIS savců se skládá z vrozené a adaptivní složky, které spolupracují v obraně proti patogenům. Vrozená složka je fylogeneticky starší (vyskytuje se u všech mnohobuněčných organismů) a poskytuje první linii obrany. Její fungování je geneticky fixované a není příliš závislé na předchozí zkušenosti. Je pro ni typická vysoká afinita k širokému spektru patogenů; reaguje nespecificky a velmi rychle. Oproti tomu fylogeneticky mladší adaptivní složka imunitního systému se objevuje až u čelistnatých obratlovců. K jejímu rozvoji u člověka dochází až po porodu a aktivace je pomalejší, neboť receptory jsou specifičtější. Zároveň dochází k budování vlastní antigenní paměti, která umožňuje rychlejší aktivaci při opětovném setkání s patogeny.

TIS je poměrně energeticky náročný, neboť po rozeznání patogenu může být spuštěna celá kaskáda metabolicky nákladných imunitních reakcí. Organismus však má k dispozici omezené množství energie, která by mohla být preferenčně využita jinde, například k reprodukci. Navíc imunitní reakce organismus obvykle oslabuje. Mnoho příznaků, jako je horečka a únava, nejsou způsobeny

patogeny, ale objevují se v důsledku boje imunitního systému s infekcí. Dá se proto očekávat vznik procesů, které omezí aktivaci TIS pouze na reálné a adekvátní hrozby.

TIS se obvykle začne aktivovat a bránit organismus až po kontaktu těla s patogenem. Bylo by však výhodnější, kdyby existovaly obranné procesy, díky nimž by se jedinec dokázal infekci vyhnout. Tuto roli by měl právě plnit BIS, který je tvořen komplexem procesů spojených s pozorností, percepcí, kognicí, afektivními stavy a chováním. Jeho hlavní funkcí je zabránit přenosu patogenů na daného jedince, ať již od někoho jiného, nebo z prostředí. Funguje tedy jako prvotní obranná bariéra organismu, která má detekovat možné nebezpečí pomocí dostupných percepčních vodítek (vizuálních, olfaktorických, akustických i taktilních), vyhodnotit jejich potenciální nebezpečnost a případně posléze aktivovat určité chování, např. vyhnutí se danému místu či člověku. Posouzení nebezpečnosti může probíhat také na základě chování ostatních, které úzce souvisí s možným přenosem patogenu, jako je např. promiskuita či manipulace se zkaženým jídlem. Na rozdíl od jiných hrozeb, patogeny (např. bakterie) obvykle není možné kvůli jejich velikosti detekovat přímo pomocí smyslů, nicméně je možné zaznamenat jejich působení, např. podle nepříjemného zápachu (zkažené jídlo) nebo vzhledu nemocného člověka. Proto musí být fungování BIS zvláště citlivé a specifické už v prvotní fázi – tedy při percepci potenciálního nebezpečí.[1]

TIS a BIS však nefungují izolovaně a mezi oběma systémy existují mnohá funkční spojení. U řady savců byla například zjištěna snížená preference nemocných jedinců (ať již v kontextu výběru partnera, nebo i při sociálním kontaktu), averzivní chování a zvýšená analgezie, které se obvykle vyskytují ve stresových nebo potenciálně nebezpečných situacích. Současně dochází ke zvýšení produkce opioidních látek, které obvykle slouží jako příprava organismu na útěk nebo boj, ale také se mohou podílet na přípravě TIS na možnou nákazu.[2] U lidí se obdobně ukázalo, že pocity znechucení vyvolávají nárůst markerů imunitních funkcí na sliznicích[3] a vystavení fotografiím s projevy infekčních onemocnění zvyšuje tvorbu prozánětlivého interleukinu 6 (Il-6) v organismu.[4]

Fungování BIS je možné vysvětlit pomocí „teorie zvládání chyb" (error management theory). Podle této teorie by systém měl být nastavený ve prospěch méně energeticky náročných chyb. V případě BIS je tak vyšší náchylnost k falešně pozitivním chybám, aby došlo ke snížení falešně negativních chyb. BIS bývá také připodobňován k principu fungování detektoru kouře, který je nastavený tak citlivě, že se spouští i při sebemenším náznaku kouře, což vede k častým falešně pozitivním chybám.[5] Tato citlivost je však vyvážena drastickým snížením falešně negativních chyb, které by mohly být fatální. I proto je relativně běžné, že BIS detekuje nějaký podnět jako potenciálně nebezpečný a spustí se vyhýbavá odpověď, přestože nehrozí reálné riziko. Lidé tak mají tendenci vyhýbat se i plastovým maketám výkalů či jídlu, které je zformováno do odpovídající podoby.[1] Takováto reakce je jen minimálně energeticky náročná, pokud by se však neaktivovala při setkání se skutečným nebezpečím, následky by mohly být velice závažné a energeticky mnohonásobně náročnější. BIS tak snižuje energetické náklady spojené s aktivací TIS.

PERCEPCE OHROŽUJÍCÍCH PODNĚTŮ

První a klíčovou funkcí BIS, aby mohla být následně spuštěna patřičná emocionální odezva – znechucení, je detekce potenciální hrozby. Detekce probíhá na základě jednotlivých smyslových modalit (vizuální, akustické, čichové i taktilní) a jejich integrace.

Vnímání podnětů jako znechucujících se v průběhu života postupně vyvíjí a jednotlivé podněty získávají valenci až na základě opakovaných interakcí. Proto jsou malé děti zpravidla tolerantní k pachu rozkladu a jemu podobným, které mohou být potenciálním zdrojem přenosu patogenů. V prvních dvou letech života je obvyklé, že si děti strkají do úst věci, které dospělí považují za nechutné, nehledě na povahu objektu a možnost nákazy. Tím se pravděpodobně učí, co jíst a co nikoliv. Od dvou let poté vnímání znechucení narůstá.[6] Při požití například něčeho hořkého nebo značně kyselého (což může indikovat něco nejedlého, ba dokonce jedovatého) však mohou vykazovat už i kojenci obvyklé mimické výrazy nelibosti, které se však liší od později projevovaného výrazu znechucení – nakrčení obočí, povytažení horního rtu a stažení nosních dírek, což může přispět k omezení vdechování potenciálně kontaminovaného vzduchu.[7]

Vizuální percepce

Nejvýznamnější modalitou, která je u člověka využívána k detekci potenciálně znechucujících podnětů, je bezesporu ta vizuální. Jedná se jak o podněty z prostředí (např. výkaly, mršiny), tak o podněty sociální. Vizuálně jsou například snadno zaznamenatelné různé morfologické anomálie, zbarvení pokožky a její kvalita, léze nebo i změny v chování jiných lidí, které mohou poukazovat na infekci, např. kýchání a smrkání. Bylo například zjištěno, že hůře byly hodnoceny fotografie, kde se vyskytoval rizikový stimul, například škrkavky (*Ascaris*), vši (*Pediculus*), otevřené rány na kůži, přeplněný dopravní prostředek apod., oproti podobným fotografiím bez rizikového stimulu. Zároveň byly zaznamenány pohlavní rozdíly ve znechucení, kdy ženy byly citlivější. Citlivost zároveň u obou pohlaví klesala se stoupajícím věkem.[8] Jak již bylo uvedeno výše, vystavení podnětům spojeným s onemocněními vzbuzuje nejen znechucení, ale podílí se i na přípravě TIS.[4] Zrak nejenže umožňuje včasné rozpoznání rizikových faktorů i na větší vzdálenost, tj. rozkládajících se těl, exkrementů či potenciálně infikovaných lidí, ale také často vede ke znechucení v důsledku přítomnosti pouze mateřských znamínek ve tváři, fyzického postižení nebo i obezity, přestože si jedinci uvědomují, že jim žádné nebezpečí nákazy nehrozí, více viz Sociální implikace.

Akustická percepce

V kontextu znechucení byly testovány zejména zvuky spojené s tělesnými procesy (např. zvracení, kašlání, odplivnutí nebo hlasité zvuky při jídle). Tyto zvuky byly hodnoceny jako velmi nepříjemné, a to opět především ženami.[9] U některých jedinců se míra nesnesitelnosti

může rozvinout až do tzv. misofonie, tj. zvýšené nelibosti k řadě zvuků, především vydávaných ostatními lidmi. Vnímání těchto zvuků vykazuje i značné kulturní rozdíly, neboť např. ve Velké Británii a Spojených státech amerických byly hodnoceny zvuky jako škrábání po tabuli či zvracení jako nechutnější nežli v dalších zemích Evropy. Při poslechu zvuků spojených se znechucením, např. zvracení či průjem, byla také zaznamenána jiná aktivita mozku nežli u zvuků vyvolávajících strach. Konkrétně se jednalo o nižší hemodynamickou odpověď v oblasti supramarginálního gyru a superiorního temporálního gyru u zvuků vyvolávajících znechucení v porovnání se zvuky vyvolávajícími strach.[10] Ukazuje se však, že změna v neurální aktivitě je v případě zvuků minimální oproti vizuálním stimulům, což může svědčit o tom, že akustická modalita není pro percepci znechucujících podnětů až tak podstatná. To podporuje i fakt, že stimuly vyvolávající silné znechucení, jako rozkládající se tělo nebo silné znečištění, jsou bez výrazného zvukového doprovodu. Podstatné jsou pak zejména zvuky přicházející od ostatních lidí či zvířat, nikoliv z okolního prostředí.

Čichová percepce

Čichová modalita je v detekci potenciální kontaminace využívána zejména ke kontrole kvality jídla (např. čicháním k mléku nebo masu). Má však i významný vliv na chemickou komunikaci mezi lidmi a do jisté míry i prověřování okolí. Místům, kde jsou cítit rozkládající se těla nebo výkaly, má valná většina lidí tendenci se vyhnout. Výhodou čichových vodítek zároveň je, že chemické látky jsou přenášeny na delší vzdálenost, a proto jsou často zaznamenány dříve, než dojde k přímému kontaktu s objektem možné nákazy. Studií zaměřujících se na konkrétní onemocnění v kontextu čichové komunikace je ale relativně málo. Z klinické praxe je však již od dob Hippokrata známo, že se různorodá onemocnění projevují typickým zápachem.[11] Výzkumy pak ukazují, že vzorky tělesné vůně od mužů aktuálně nakažených kapavkou i od jedinců s aktivovanou imunitní odpovědí po injikaci lipopolysacharidu byly hodnoceny jako méně příjemné a zdravé. Lze předpokládat, i když uvedené studie tuto otázku přímo netestují, že by se ostatní jedinci nemocným spíše vyhnuli, když je jsou schopni detekovat a hedonicky odlišit od zdravých. U participantů, kteří byli vystaveni tělesné vůni jedinců, u nichž bylo znechucení navozeno pomocí videa, byly zaznamenány mimické výrazy znechucení (tj. nakrčený nos, povytažený horní ret a zúžené zorné pole – nižší míra rozhlížení se).[12] Navíc se ukázalo, že při současné prezentaci tělesné vůně a fotografií snižovala tělesná vůně nemocných hodnocení atraktivity tváří.[13]

Taktilní percepce

Kůže funguje jako prvotní bariéra před patogeny. I proto může setkání se znechucujícím podnětem vést kupříkladu ke zvýšené potřebě hygieny, aby bylo zabráněno nákaze. Dotykem můžeme detekovat vlhkost, teplotu i soudržnost daného podnětu (např. plody a rostliny jsou typicky křehčí, když jsou napadené patogeny, neboť ty se podílejí na rozpadu biologické tkáně). Ve studii zaměřené na detekci podnětů hmatem se ukázalo, že vlhké stimuly byly hodnoceny jako významně více znechucující a méně příjemné než suché stimuly, stejně tak měkké (těstovité) podněty byly hodnoceny jako nechutnější nežli podněty pevné. Je tak patrné, že vlhkost a nízká soudržnost jsou často vnímány jako nechutné, kvůli možnému výskytu patogenů.[14] Přítomnost znechucujícího vizuálního podnětu navíc zvyšuje taktilní senzitivitu měřenou testem Semmes-Weinsteinových monofilament. V tomto testu jsou využívána nylonová vlákna o postupně se zvyšující šířce/hmotnosti, dokud nejsou na kůži daným jedincem vnímatelná. Zvýšená taktilní senzitivita v důsledku vystavení vizuálním podnětům vede k vyšší tendenci se čistit v porovnání s neutrálními stimuly a stimuly vyvolávajícími strach.[15]

Integrace smyslů

V běžném životě je člověk vystaven podnětům, které působí na více smyslů současně a vedou k jejich integraci a ovlivnění zpracování informace z jednoho smyslu modalitami dalšími. Propojení smyslů je důležité i proto, abychom s vyšší pravděpodobností zaznamenali, co se v našem okolí děje, a mohli snáze vyhodnotit potenciální nebezpečnost dílčích stimulů. Na jednu stranu může docházet k umocňování odpovědi (*multisensory enhacement*), nebo také k poklesu odpovědi (*multisensory depression*). V případě znechucení nejčastěji dochází k propojení vizuální a čichové modality. Další modalita zpravidla moduluje či zesiluje percepci té první, např. nepříjemná vůně snižuje hodnocení atraktivity tváří či při prezentaci znechucujícího vizuálního podnětu se zvyšuje hmatová citlivost. Podobně mohou určité barvy ovlivnit hodnocení zvuků (např. modrá a zelená negativně ovlivňovaly vnímání nepříjemnosti a znechucení poslouchaného zvuku[16]). Kombinace smyslových vjemů tak poskytuje efektivnější a přesnější zpracování smyslového vjemu a následnou reakci na něj. Zároveň také dochází ke zvýšení pozornosti ostatních smyslů při prvotním zaznamenání podnětu. Další výzkumy zaměřené na testování BIS a znechucení by se měly zaměřit na propojení modalit a jejich vzájemné ovlivňování, neboť v běžném životě není vnímání podnětů omezeno pouze na jeden či dva smysly. Multimodální prezentace znechucujících stimulů zároveň umožní testovat jejich relativní důležitost v rámci BIS.

KOGNITIVNÍ SLOŽKA BIS

Kognitivní složka BIS nám umožňuje uvědomovat si a zvažovat míru nebezpečnosti při setkání s potenciálně rizikovým podnětem, zvířetem či člověkem. Jinak je tomu u mladších dětí, které nemusejí rozumět tomu, co nebo kdo může představovat nebezpečí s ohledem na kontaminaci patogeny. Pravděpodobně tak existuje určitá kognitivní hranice u mladších dětí, které nejsou schopny jednoznačně rozlišit, zdali je daný objekt nebezpečný a/nebo kontaminovaný. Například až po osmém roce života je dítě schopné říct, že určitý objekt je ten samý po odstranění znechucujícího podnětu z jeho povrchu, např. výkalu či hmyzu, potažmo že hrozí možná nákaza i po „očištění".[6]

Do této oblasti by spadaly i kulturní normy snižující možnost infekce. Nicméně celá řada kulturních pravidel či zvyklostí je dodržována bez ohledu na to, zda jsou v dané kultuře spojovány se snížením rizika přenosu. Naopak mohou být součástí náboženských či magických představ, které s infekcí nemusejí vůbec souviset. Mezikulturně jsou například mnohé činy spojené s rizikem kontaminace patogeny brány jako nepřijatelné a nemorální. Příkladem může být zákaz nebo omezení požívání určitého jídla (zejména živočišných produktů, např. masa), zoofilie, prostituce nebo i zacházení s tělesnými tekutinami a výměšky. Existuje však také mezikulturní variabilita, která je do určité míry závislá na riziku kontaminace – čím větší možnost nakažení se, tím zpravidla vyšší míra znechucení a odsuzování určitého chování, které k nákaze může vést. Na flexibilitu nejen kognitivní složky BIS také poukazuje situace např. při epidemiích či pandemiích, kdy se lidé vyhýbají více přelidněným prostorům a více dodržují hygienická pravidla.[17]

AFEKTIVNÍ SLOŽKA BIS

Jedním z klíčových mechanismů, které by měly u člověka zajistit snižování pravděpodobnosti nákazy infekcí, je emoce znechucení (*disgust*), kterou tvoří soubor kognitivních, behaviorálních a fyziologických procesů.[18] Znechucení se vyznačuje pociťovaným odporem, někdy doprovázeným nevolností, a především silnou touhou se od podnětu co nejdříve vzdálit. Zároveň může být doprovázeno specifickým mimickým výrazem znechucení (nakrčení nosu a vytažení horního rtu), zpomalením srdečního tepu, změnami kožní vodivosti, a dokonce mobilizací tělesného imunitního systému.[19,20] Někteří autoři předpokládají, že znechucení se skládá z patogenní domény a odvozené sexuální a morální domény.[21]

Patogenní znechucení

Starší pojetí chápala znechucení převážně jako způsob, jak se vyhnout požití něčeho nejedlého.[20] Novější, evolučně psychologické koncepce nahlížejí na znechucení jako na adaptaci, která se vyvinula za účelem prevence nákazy infekčními onemocněními, což je možné nejen požitím něčeho zkaženého, ale také skrze tělesné tekutiny, výměšky (krev, zvratky, ejakulát, fekálie) a ostatky, které mohou obsahovat značné množství patogenů.[8,18,21] Patogenní znechucení (*patogen disgust*) je tak považováno za základní stavební kámen emoce znechucení.[18] Dá se proto očekávat, že znechucení bude: I) snadno vyvoláno situací, ve které je možné se nakazit onemocněním; II) obdobně působit napříč kulturami; III) silnější u žen, které chrání před nakažením jednak sebe, jednak případného potomka; IV) snižovat se s věkem, tedy společně s reprodukčním potenciálem; V) vyvoláno spíše kontaktem s cizinci než s blízkými, jelikož cizinci mohou být spíše nositeli neznámých patogenů. Tyto předpoklady byly podpořeny ve studii Curtis et al.[8] Participanti ve výzkumu mj. označovali jako více znechucující fotografie, na kterých byly zachycené tělesné tekutiny (vs. modré barvivo), obličej člověka, který vypadá nemocně a je zpocený a zarudlý (vs. obličej zdravého jedince), příp. fotografie zaplněného (vs. prázdného) metra.[8]

Sexuální znechucení

Sexuální znechucení (*sexual disgust*) je negativní afektivní reakce na určité sexuální partnery a praktiky. Očekává se, že sexuální znechucení se bude primárně týkat jedinců a aktivit, které mohou ohrožovat dlouhodobý reprodukční úspěch.[21] Řada studií prováděných na savcích a ptácích zejména v kontextu pohlavního výběru ukazuje na vyhýbavé chování vůči nemocným či nepříliš zdatným jedincům. Sexuální znechucení se obvykle projevuje jako reakce na nechtěný sexuální kontakt a je o něm možné uvažovat jako o protikladu sexuálního vzrušení.[21] Senzitivita žen v této doméně je oproti mužům vyšší, zřejmě kvůli energetické náročnosti spojené s (nechtěným) těhotenstvím, péči o potomka či s hrozbou nákazy pohlavně přenosnými nemocemi.[8,21] Sexuální znechucení se oproti patogennímu liší v tom, jaké informace při vytváření úsudků bereme v potaz. Mnoho z nich není relevantních pro vyhýbání se patogenům, ačkoli se s touto doménou často překrývají. Většině lidí například připadá nechutná představa sexu mezi blízce příbuznými jedinci. Vyhýbání se by také mělo být specifické pro sexuální aktivity. Pokud daný jedinec není vhodný jako sexuální partner, není nutné se vyhýbat i dalším sociálním interakcím s ním (např. přátelství).[21]

Morální znechucení

Morální znechucení (*moral disgust*) je negativní afektivní reakce na porušení společenské normy. Tato doména sice využívá stejné afektivní mechanismy, nicméně funkčně se zřejmě jedná o odvozený proces. Morální znechucení nesouvisí přímo s BIS, ale mohlo by souviset s konformismem vůči společenským normám obecně, jejichž porušování může zvyšovat riziko přenosu patogenů. Jako znechucující je vnímáno antisociální chování, např. lhaní, podvádění a krádež.[21] Rozpoznat jedince, kteří porušují normy, a vyhnout se jim udržuje skupinovou kohezi a kooperativní vztahy. Někteří autoři namítají, že společenské přestupky nemusejí vyvolávat pocit znechucení *per se* a jde pouze o rétorický či metaforický popis.[20,22] Studie využívající funkční magnetickou rezonanci ukázaly, že morální znechucení a patogenní znechucení vyvolávají aktivace jak ve shodných mozkových regionech (např. bazální ganglia, amygdala, talamus), tak i v odlišných oblastech mozku (v případě morálního znechucení se jednalo např. o aktivaci střední části prefrontálního kortexu, části temporálního laloku a precunea).[22]

METODY MĚŘENÍ BIS

K měření různých složek BIS, potažmo znechucení, je využíváno poměrně široké spektrum metod. Hlavní oblasti zahrnují testování rozdílů v charakteristikách spojených s BIS a jejich souvislost s dalšími charakteristikami jedinců pomocí dotazníkového šetření a experimentální manipulace s podněty vyvolávajícími znechucení.

Interindividuální rozdíly v charakteristikách spojených s BIS a jejich vztah s dalšími charakteristikami (např. osobnost) jedinců jsou obvykle měřeny pomocí osobnostních testů a dotazníků. K nejrozšířenějším nástrojům patří Vnímaná náchylnost k nemocem (*Perceived Vulnerability to Disease scale, PVD*) měřící předsudky vůči handicapovaným a imigrantům, novější verze se pak týká vyhýbání se patogenům a náchylnosti k onemocnění. Dotazník znechucení (*Disgust scale, DS*) zahrnuje 8 oblastí možných spouštěčů znechucení – jídlo, živočichové, tělesné výměšky, porušení tělesné schránky, sex, smrt, hygiena a později doplněná oblast zpracovávající tzv. sympatetickou magii (přenos určitých vlastností na základě vnější podoby).[23] Revidovaná verze dotazníku znechucení (*Disgust Scale – revised, DS-R*), sestává ze 3 kategorií – základní (znechucení z možného přenosu patogenu), odpor vycházející z animálního původu člověka a strachu z kontaminace.[24] Přestože se první a třetí kategorie částečně překrývají, jedná se o jeden z nejvyužívanějších dotazníků a je k dispozici i v české jazykové mutaci.[25] Jak již z názvu vyplývá, také Třídoménový dotazník znechucení (*The Three-Domain Disgust Scale, TDDS*) rozděluje znechucení do tří domén, avšak tentokrát na patogenní, sexuální a morální.[21] V budoucnu by se měla pozornost zaměřit na rozdíly mezi jednotlivými dotazníky v tom, jaké aspekty znechucení měří, a pro testování daných hypotéz používat vždy nejvalidnější nástroje. V tuto chvíli se totiž zdá, že bývají vybírány poměrně volně a pracuje se s nimi, jako by byly zaměnitelné.

Znechucení založené na vnímání pachů specificky měří Dotazník znechucení z tělesných pachů (*The Body Odor Disgust Scale, BODS*). Zaměřuje se na různé zdroje tělesného pachu – dech, horní polovina těla, nohy, moč, výkaly, genitálie a plyny z trávicího ústrojí – a to jak pachu vlastního, tak pocházejícího od ostatních jedinců.[26]

Oproti dotazníkům postaveným na tvrzeních by silnější reakce měly vyvolávat vizuální podněty. Proto Curtis et al.[8] vytvořili sedm párů fotografií, z nichž jedna obsahovala potenciální zdroj nákazy, zatímco druhá nikoliv. Tato sada fotografií je však metodicky problematická (např. některé páry fotografií se liší nejen klíčovým podnětem, ale i dalšími detaily), a navíc nepokrývá některé situace možné infekce (např. výkaly či rozkládající se těla). Byl proto vytvořen nový nástroj – Culpepperova sada znechucujících snímků (*Culpepper Disgust Image Set, C-DIS*), který prošel extenzivní validizací a jehož výsledná verze sestává z 20 párů fotografií, vždy s jedním stimulem vzbuzujícím znechucení a druhým komplementárním kontrolním snímkem.[18] Všechny výše uvedené dotazníky (BDDS, BODS), tak i sada snímků (C-DIS) jsou k dispozici v české jazykové verzi na požádání od autorů.

Experimentální přístup spočívá ve vystavení participantů potenciálně patogenním stimulům a následném měření jejich postojů či chování. Ke stimulaci BIS jsou využívány různé druhy stimulů, a to čichové, vizuální (někdy doprovázené textem popisujícím riziko infekce), zvukové, taktilní či krátké popisy možného kontaktu s patogeny. V současnosti je využívána i virtuální realita, která byla například úspěšně použita k navození znechucení z kontaminovaného jídla.[27] Různé typy stimulů však nemusí BIS zpracovávat stejným způsobem a síla a typ reakce se tak mohou lišit. Lze předpokládat, že kupříkladu taktilní stimuly kvůli přímému kontaktu se zdrojem nákazy budou vzbuzovat silnější vyhýbavou reakci než podněty čichové.

Míru znechucení je také možno nepřímo měřit prostřednictvím fyziologických reakcí na určitý podnět. Ukázalo se například, že při vystavení znechucujícím stimulům dochází k mírnému zpomalení srdečního tepu a zvyšuje se kožní vodivost (měřítko sympatické autonomní aktivity spojené se vzrušením a orientací ke stimulům nesoucím podstatnou informaci).[28] V obou případech se však jedná o nespecifické reakce, které jsou vyvolány i jinými než znechucujícími podněty. Ke specifické aktivaci dochází u mimických svalů spojených s výrazy znechucení (zejména musculus levator labii superioris, který se podílí na zdvižení horního rtu), jež jsou měřené elektromyografií, či je možné reakce jedince nahrávat na videozáznam a následně kódovat jeho chování pomocí etogramu.

SOCIÁLNÍ IMPLIKACE

BIS ovlivňuje chování jedinců tak, aby se minimalizovala rizika přenosu infekcí. Nicméně v poslední době se ukazuje, že tyto procesy mohou ovlivňovat různé aspekty společenských interakcí včetně postojů k sexuálnímu chování, vnímání ostatních jedinců, společenskosti, xenofobii či konformitě, a to jak na úrovni jednotlivců, tak celých populací. V souladu s „teorií zvládání chyb" (viz výše) můžeme očekávat vyšší množství falešně pozitivních chyb, jejichž následky jsou v porovnání s falešně negativními chybami mnohem menší. V důsledku tak mohou lidé reagovat nejen na podněty, které představují riziko infekce, ale i na bezpečné podněty, jež s nimi pouze sdílejí určitou podobnost. Tato generalizace vede k předsudkům vůči jedincům, jejichž vzhled se nějakým způsobem podobá jedincům trpícím infekcí (i když s infekcí a jejím přenosem vůbec souviset nemusí). Podobně generalizace vede k předsudkům vůči jedincům, kteří se nějakým způsobem odchylují od normy nebo porušují normy, které souvisí s rizikem přenosu patogenů.[29] Následně může docházet k jejich stigmatizaci. Lidé nemocní nebo vykazující znaky běžně spojené s nemocí (např. pokleslá víčka, červené oči) bývají hodnoceni jako méně společensky žádoucí. Zmíněné znaky se však velmi často vyskytují i při pouhé únavě. V implicitním asociačním testu byly tváře mužů s mateřským znaménkem častěji spojovány s onemocněním, i když byli popsáni jako zdraví, oproti mužům bez mateřského znaménka, u kterých bylo uvedeno, že trpí tuberkulózou. Podobně lidé raději udržují větší fyzickou vzdálenost od jedinců, kteří jsou nemocní, a to zvláště, pokud je nemoc, byť jen zdánlivě, nakažlivá.[29]

Dosavadní výzkumy se často zabývají vztahy mezi morfologickými odchylkami od normy a aktivací BIS. Mezi takové patří například stárnutí, kdy se člověk s přibývajícím věkem stále více odlišuje od vzhledu mladších lidí a snižuje se obranyschopnost jeho organismu, což může u ostatních jedinců BIS aktivovat. Zvláště náchylní jsou k tzv. ageismu (předsudkům vůči starým lidem) lidé, kteří se domnívají, že se mohou snadno nemocemi nakazit.[30] Společenské dopady tzv. ageismu (předsudkům vůči starým lidem) jsou značné. Starší lidé spíše neuspějí, pokud se o stejnou pracovní pozici uchází někdo mladší, a v domácnosti jim spíše hrozí zanedbání a týrání než

lidem mladším.[30] Analogicky se některé studie zaměřují na obezitu.[31] I zde platí, že lidé s většími obavami z nákazy infekčním onemocněním mají vůči obézním negativnější postoje. V tomto případě je však otázkou, do jaké míry je tento jev kulturně specifický pro euro-americké prostředí, protože v celé řadě tradičních společností je obezita spojována s vyšším společenským statusem. Handicapovaní jedinci rovněž vykazují určité fyzické anomálie, přičemž BIS nerozlišuje mezi chybějící končetinou způsobenou infekční nemocí nebo nešťastnou nehodou.[32]

BIS bývá spojován i s xenofobií. Podle některých studií[1] mohou jedinci mimo skupinu, kterou vnímáme jako vlastní, (tzv. outgroup jedinci) aktivovat tento systém svou fyzickou odlišností, která je BIS vyhodnocena jako odchylka od normy. Kontaktem s těmito jedinci může teoreticky docházet k přenosu exotických patogenů, které mohou být pro místní populaci vysoce virulentní. V neposlední řadě mohou outgroup jedinci porušovat lokální kulturní normy, což zvyšuje riziko infekce v celé skupině, jelikož tyto normy často slouží ke snížení pravděpodobnosti přenosu patogenů. Riziko se totiž mění nejen podle toho, jak se chová daný jedinec, ale jak se chovají i lidé v okolí.

Podobně i některé kulturní normy, tradice a rituály mohou vést ke snížení rizika onemocnění (např. normy v oblasti přípravy jídla, osobní hygieny, sexuality).[1,29] I v tomto případě nezáleží pouze na dodržování norem daným jedincem, ale i ostatními. Snížení rizika infekce tak zahrnuje odměňování konformistů a přísné postihy nekonformních jedinců. Větší tendence podléhat konformitě by se měly objevovat v případech s vyšší pravděpodobností přenosu onemocnění. Několik studií skutečně ukázalo, že lidé jsou v takových situacích méně přístupní novým myšlenkám a zkušenostem a jsou přísnější vůči jedincům, kteří porušují společenské normy.[29]

Obdobnou situaci můžeme najít i na úrovni celých populací. V oblastech s historicky větší prevalencí parazitů lidé vykazují vyšší tendence ke konformitě a jejímu vynucování, větší míru xenofobie či menší míru extraverze. Někteří autoři se domnívají, že výskyt patogenů a obavy z nich ovlivňují výskyt kolektivismu vs. individualismu v různých geografických oblastech, kdy v oblastech s historicky vyšší prevalencí infekčních nemocí jsou kultury spíše kolektivistické.[1]

Funkční flexibilita

BIS je funkčně flexibilní a jeho fungování je spojeno s celou řadou interindividuálních a intraindividuálních rozdílů. Obecně je silnější averzní reakce vůči potenciálně rizikovým podnětům možné sledovat v situacích, kdy je vyšší nebezpečí infekce, anebo kdy si jedinec myslí, že může být daná situace riziková.[31]

Například ženy v prvním trimestru těhotenství vykazovaly vyšší míru znechucení a silnější etnocentrické a xenofobní postoje nežli ženy v pozdějších fázích těhotenství a stejný efekt se ukázal i poté, co byl vzat v úvahu výskyt těhotenské nevolnosti. S tím souvisí tzv. kompenzační profylaktická hypotéza, která předpokládá zvýšenou míru znechucení se sníženou imunokompetencí. V prvním trimestru dochází v TIS u těhotné ženy ke komplexním změnám především kvůli toleranci imunitně cizorodého plodu. Zároveň je plod velice náchylný k působení teratogenů, proto se předpokládá, že by BIS mohl být jedním z hlavních obranných mechanismů.[33]

Některé osobnostní rysy vykazují spojitost s fungováním BIS. Společenštější jedinci (extravertnější) jsou více ohroženi potenciálním onemocněním kvůli kontaktu s větším množstvím lidí. Naopak lidé, kteří se cítí být náchylní k onemocnění, mohou být méně společenští, mít méně přátel a známých.[1] V důsledku nižší míry sociálních kontaktů pak ale tito lidé mohou trpět nedostatečnou podporou, osamělostí a vyšší mírou kumulativního stresu.

Aktivace BIS je dále vázaná na intraindividuální rozdíly, což je například aktuální i minulý zdravotní stav jedince. Bylo například zjištěno, že jedinci, kteří v posledním týdnu prodělali infekční onemocnění (jejich TIS byl aktivován), rychleji a výrazněji reagovali na podněty spojené s možnou nákazou.[31] Podobně i prostředí a kontext významně ovlivňuje aktivaci a citlivost BIS. Například u zdravotníků pracujících na infekčním oddělení, nebo u matek pečujících o nemocné děti bude i v první obranné vlně spíše upřednostněn TIS před BIS, který v takovémto prostředí už není efektivní.[34]

BIS a pandemie COVID-19

Při přípravě tohoto textu jsme netušili, že jej budeme dokončovat v době vrcholící pandemie nového koronaviru SARS-COV-2, způsobujícího onemocnění COVID-19, který se objevil v čínské provincii Wuhan na konci roku 2019. Celá řada jevů, které současnou pandemii doprovázejí, nápadně připomíná výsledky studií testujících aktivaci BIS v laboratorních podmínkách. Nyní jsme však jejich svědky na celoplanetární úrovni. Níže uvádíme příklady některých jevů souvisejících s BIS zdokumentovaných v odborném tisku.

Již několik týdnů jsme kvůli současné pandemii pod tlakem, který může značně zvyšovat úzkost a mít dopad na naše duševní zdraví. Zároveň se začaly objevovat zprávy o zvýšení míry xenofobie, kdy dochází k verbálním i fyzickým útokům na jedince asijského původu.[35] Z Bangladéše a Indie byly hlášeny případy, kdy muž vracející se z města zpět do rodné vesnice spáchal sebevraždu, protože se její obyvatelé obávali, že je nakažený koronavirem.[36] Zbrusu nové výzkumy pak ukazují, že předsudky vůči cizincům jsou spojené s množstvím sledování médií (tedy vystavení informacím o koronaviru), a nejvíce se v dané studii objevily vůči Italům, kteří byli v době vzniku studie nejpostiženější zemí v Evropě.[37] Lidé se také stávají konformnějšími a vyhledávají stabilitu a pořádek. Ve volbách prezidenta v USA a Polsku se v současném stavu pandemie přiklánějí ke konzervativnějším kandidátům.[38] Tyto reakce je možné sledovat především u úzkostlivější lidí. Podobně bylo již dříve zjištěno, že v dobách ohrožení mají lidé tendenci volit kandidáty s maskulinnějšími obličeji.[39]

V tuto chvíli lze jen těžko odhadovat, zda se po odeznění pandemie naše společenské zvyklosti vrátí k původnímu stavu, nebo bude mít hromadná aktivace BIS dlouhodobější dopad (např. udržování osobních rozestupů, nedotýkání se při pozdravu apod.). V současnosti provádíme výzkum, v němž jsme se zaměřili právě na aktivaci různých aspektů BIS (např. úzkost, znechucení, xenofobie, hygienické návyky) za probíhající pandemie a následně

budeme testování opakovat po jejím zklidnění. Poté snad budeme moci na tyto otázky alespoň částečně odpovědět.

ROLE BIS PŘI VZNIKU NĚKTERÝCH PSYCHIATRICKÝCH PORUCH

Hlavní funkcí BIS je u zdravého jedince zabránit organismu v kontaktu s rizikovými faktory a snížit pravděpodobnost přenosu patogenů. Pokud však dochází k jeho opakované aktivaci v neadekvátních situacích, může se podílet na vzniku některých psychiatrických onemocnění. Řada studií poukazuje na souvislost mezi znechucením a úzkostnými psychiatrickými poruchami, kupříkladu fóbie ze zvířat, obsedantně-kompulzivní poruchy (OCD) nebo strach z onemocnění a lékařských zákroků.[40] V současnosti však není jasné, zda znechucení ovlivňuje vznik těchto poruch, či naopak tyto poruchy zvyšují citlivost ke znechucení. Velmi vysokou prevalenci má zejména OCD (zhruba 2,3 % populace), při níž je reaktivita organismu na okolní podněty vyšší a často neúměrná reálnému riziku.[41] Jedná se o jedno z nejčastějších psychiatrických onemocnění, které postihuje obdobně často ženy i muže a může se vyskytovat u dětí, adolescentů i dospělých. OCD způsobuje silné úzkosti, obavy a napětí z opakujících se myšlenek (obsese), které velmi často ústí v určitý vzorec chování, jenž má podobu opakujících se úkonů (kompulze).

Až polovina OCD přitom souvisí se strachem z kontaminace, což vede k vyhýbání se nejen stimulům, ale také situacím spojeným s možným přenosem infekce či silným znečištěním. Ústřední roli zde hraje znechucení, tedy emoční proces, který ovlivňuje náš přístup k potenciálně nebezpečným podnětům. Ukazuje se, že tato emoce je lepším prediktorem obsedantně-kompulzivní poruchy v souvislosti s kontaminací než např. úzkost, strach nebo deprese.[42] U jedinců vykazujících silné symptomy OCD bylo zjištěno, že mají výrazně vyšší citlivost ke znechucení při sledování videa s pohybujícími se larvami v otevřené ráně i při vyplňování dotazníku zaměřeného na znechucení než jedinci, kteří tyto symptomy nevykazovali; efekt se ukázal i při kontrole efektu strachu.[43] Když byly v experimentu otírány tužky o kontaminovaný předmět a posléze vždy čistá tužka otřena o předtím využitou tužku, ukázalo se, že i po 12 opakováních se jedincům s OCD snížilo vnímání kontaminace poslední využité tužky jen o 40 % oproti kontrolním jedincům, kteří hlásili snížení o 100 %.[44]

Několik studií se zaměřilo na identifikaci neurálních korelátů souvisejících se znechucením u pacientů s OCD, výsledky jsou však poněkud nejednoznačné. Při pohledu na fotografie tváří znechucených jedinců byla u jedinců s OCD zjištěna vyšší aktivace levé části ventrolaterárního prefrontálního kortexu, ale snížená aktivace talamu oproti zdravým kontrolám; u vystrašených tváří nebyl mezi skupinami nalezen rozdíl. Některé studie zjistily silnou aktivaci inzuly, ale nikoliv amygdaly, při prezentaci obrázků souvisejících se znečištěním a možnou kontaminací pacientů s OCD, v jiných se naopak ukázala výraznější aktivace amygdaly. V další studii však žádná silnější odezva těchto mozkových struktur oproti kontrolním jedincům nebyla nalezena. Novější studie pak ukazují, že u OCD související se strachem z kontaminace dochází k abnormální aktivaci v oblasti inzuly a ventrolaterárního prefrontálního kortexu při kontaktu se stimuly spojenými s možností kontaminace. Tyto oblasti mozku také mohou být propojeny se senzitivitou ke znechucení. Interpretaci výsledků však znesnadňuje výběr výzkumného vzorku, neboť v některých studiích jsou využívány heterogenní skupiny různých typů OCD.[43,45]

Znechucení je tak emocí, která je klíčovou jak u BIS, tak i u OCD. V případě tohoto onemocnění se tak může jednat o poruchu BIS, který reaguje zvýšenou citlivostí a neúměrnou odezvou na neadekvátní stimuly, ústící až ke vzniku psychiatrických poruch. I proto by se budoucí výzkum měl zabývat souvislostmi mezi BIS a OCD, nejenom jejich společným mediátorem – znechucením.

ZÁVĚR

BIS představuje první ochrannou „bariéru“ organismu, která sestává z řady psychologických mechanismů, jež mají za úkol zabránit možné kontaminaci patogeny. Detekce potenciálních ohrožujících podnětů probíhá na základě vizuálních, olfaktorických, akustických i taktilních vodítek, načež může být aktivována emocionální odpověď – znechucení. Kaskáda percepce, kognice a následné emocionální odpovědi na základě znechucujícího podnětu může vést až k vyhýbavému chování. BIS je tak složkou, která může ušetřit energii, jež by musela být organismem vydána v případě nakažení a aktivace TIS. Pro dostatečnou efektivitu systému však musí být citlivost BIS velmi vysoká, což v důsledku může vést k řadě falešně pozitivních chyb, které se ukazují v běžném životě jako řada sociálních předsudků, např. vůči cizincům. Odchylky od standardního fungování BIS také můžeme zaznamenat v případě jedinců trpících OCD, zejména u typu s rozvinutým strachem z kontaminace. Přestože BIS je poměrně novým konceptem, extenzivní výzkum v posledním desetiletí poukazuje na jeho význam a dopad na mnohé aspekty lidského života.

Poděkování
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Statement of contribution

This is to confirm that Mgr. Dagmar Schwambergová significantly contributed to the following publication:

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

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doc. Mgr. Jan Havlíček, Ph.D.
supervisor

Chapter 8

PANDEMIC ELEVATES SENSITIVITY TO MORAL DISGUST BUT NOT PATHOGEN DISGUST

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Pandemic elevates sensitivity to moral disgust but not pathogen disgust

Dagmar Schwambergová[1,2]✉, Šárka Kaňková[3], Jitka Třebická Fialová[1], Jana Hlaváčová[3] & Jan Havlíček[1,2]

The behavioral immune system, with disgust as its motivational part, serves as the first line of defense in organisms' protection against pathogens. Laboratory studies indicate that disgust sensitivity adaptively adjusts to simulated environmental threat, but whether disgust levels similarly change in response to real-life threats, such as a pandemic, remains largely unknown. In a preregistered within-subject study, we tested whether the threat posed by the Covid-19 pandemic would lead to increased perceived disgust. The perception of threat was induced by testing during two phases of the Covid-19 pandemic (periods of high vs. low pathogen threat). We found heightened levels of moral disgust during a "wave" of the pandemic, but the effect was not observed in the domain of pathogen or sexual disgust. Moreover, the age of respondents and levels of trait anxiety were positively associated with pathogen and moral disgust, suggesting that variation in disgust sensitivity may be based chiefly on stable characteristics.

Immunological defense plays a crucial role in our resistance against pathogens, but the physiological immune system is also energetically costly and metabolically demanding. These demands can be reduced by activation of a behavioral immune system (BIS), which consists of psychological mechanisms directed at pathogen detection. They activate an affective and cognitive response which may lead to pathogen avoidance and hygienic behavior aimed at minimizing contagion risks[1]. The motivational (affective) component of the BIS is disgust[2], frequently accompanied by physiological responses, such as nausea or lowered blood pressure[3]. Tybur and colleagues[4] proposed that disgust consists of three functionally specific domains: moral (motivates avoidance of violators of social norms), sexual (motivates avoidance of sexual partners and behaviors which would jeopardize one's long-term reproductive success), and pathogen disgust. The last-named is the most crucial domain for avoidance of possible contagion. While pathogen avoidance is a cross-cultural phenomenon and detection of cues to sickness is not restricted to in-group individuals[5], there is a high interindividual and intraindividual variation in disgust sensitivity, influenced by numerous factors including gender[6], age[6,7], reproductive status[8], and immunological vulnerability[9].

Disgust sensitivity varies also depending on external factors, such as environmental risks. For example, a recent study by Cepon-Robins et al.[10] showed that in Ecuadorian Shuar communities, which live in an environment with a high pathogen risk, higher pathogen disgust sensitivity led to lower infection levels. But even in generally low pathogen risk environments, epidemics do occur, and the BIS is expected to react flexibly to protect the organism. The Covid-19 pandemic, which currently poses a serious global threat[11], represents such a situation, and emerging evidence is showing activation of the BIS via elevated disgust sensitivity. For example, Milkowska et al.[12] compared disgust sensitivity in Polish women before and during the pandemic: during the pandemic, respondents reported higher disgust sensitivity to disgust-evoking pictures, but such increase was not observed in the pathogen domain of the Three-Domain Disgust Scale (TDDS). In another study, the germ aversion subscale of the Perceived Vulnerability to Disease Questionnaire (PVD) and the pathogen domain of TDDS were strongly associated with Covid-19 concerns and preventive behaviors such as social distancing, mask wearing, and cleaning[13]. Moreover, Australian students who completed a questionnaire during a lockdown reported higher disgust sensitivity than students who completed the same questionnaire before the pandemic[14]. All existing research, however, compares the scores of different groups of participants and comparability of the

[1]Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic. [2]National Institute of Mental Health, Topolová 748, 250 67 Klecany, Czech Republic. [3]Department of Philosophy and History of Science, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic. ✉email: schwambd@natur.cuni.cz

groups might be an issue. There is, therefore, a need to study this issue using a within-subject design that would monitor intraindividual fluctuations depending on the level of pathogen threat.

Gender and age seem to be the most prominent variables influencing disgust sensitivity. While the results for gender are unequivocal, with women showing on average higher disgust sensitivity than men do[6,15], the effect of age is far less clear. For instance, Curtis et al.[6] showed images with a potential disease threat to participants and found that the level of disgust was decreasing with age. Similarly, older participants scored lower on disgust sensitivity[7]. Other studies, however, report a positive association between both age and food disgust[16] and age and germ aversion, although perceived infectability decreased up to the age of fifty[15].

This study's aim was to test whether the threat caused by the Covid-19 pandemic leads to increased perceived disgust as a major variable linked to the BIS. We employed a mixed research strategy combining an experimental design (a priming vignette, which is a common tool used in research on sexual and moral disgust[17–19]) and a naturally occurring event (the Covid-19 pandemic). The perception of threat was experimentally induced by a priming story (between-subject design). Each participant was tested twice (within-subject design): once during the first lockdown (high pathogen threat) and once over a year after the onset of pandemic, at a time when restrictions were lifted (low pathogen threat). Based on data from the National Institute of Public Health, we know that in the Czech Republic, the 21-day cumulative number of reported Covid-19 cases per 100,000 persons was 44 during the period of high pathogen threat and a strict lockdown (with 115–377 new cases a day in March–April 2020) and 25 during the period of low pathogen threat after most restrictions were lifted (with 41–208 new cases a day in June–July 2021). In line with preregistration, we expected higher scores in pathogen and moral disgust during the high pathogen threat period irrespectively of the priming. We also predicted that experimental priming would elevate the scores of disgust measures during the period of low, but not high pathogen threat due to a predicted ceiling effect. We expected no changes in trait anxiety, or general health status between the two periods but predicted that scores of trait anxiety and health-related questionnaire scores would positively modulate changes in disgust. Based on data from our pilot study, we also expected age to positively correlate with the pathogen and moral disgust domains during the period of high pathogen threat but not during the low pathogen threat.

Materials and methods

The project was conducted online as a prospective study in the Czech Republic and included two data collection points at which participants were exposed to the same (experimental or control) priming conditions and completed the same set of questionnaires. The study was approved by the Institutional Review Board of Charles University (approval number 2020/02) and had been performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants. Our predictions and the design were preregistered prior to accessing the data (https://osf.io/tvmxg).

Power analysis. Statistical power analysis was computed in G*Power (Version 3.1)[20]. It was a priori specified that we would need a sample size of 180 participants to detect a medium effect size (d = 0.53; computed as a mean of effect sizes from relevant studies[21–23]) with an alpha of 0.05 at both data collection points. Based on our previous studies, we estimated that around 50% of respondents from the first round would be willing to participate in the second one (i.e., provide their email address). We further expected that around 75% of those who provide the email address would complete the study. Our target was thus to recruit at least 700 participants in the first round to have around 260 participants in the second round.

Participants. We recruited participants (aged over 18 years) using a snowball sampling method. Respondents were contacted online, via Facebook sites and emails, and invited to participate in a study called Personality and Disgust. A total of 1047 respondents took part in the first round but 286 individuals were excluded from further analyses, mostly because they failed to complete the questionnaire (over one-fifth of the questionnaire unanswered). This resulted in a final sample size of 761 respondents (586 women and 175 men; women: $M_{age} = 33.1$, SD = 11.8; men: $M_{age} = 33.4$, SD = 10.6) in the first round, i.e. during the first "wave" of the Covid-19 pandemic. Fourteen months later, we recontacted participants who expressed interest in further participation and provided their email addresses (N = 442). In the second round, 249 participants took part but 25 were excluded because they did not complete the questionnaire. This resulted in a final sample size of 224 participants (175 women and 49 men; women: $M_{age} = 33.0$, SD = 10.9; men: $M_{age} = 33.4$, SD = 12.4). Participation in the study was conditional on informed consent and no financial reward was offered to respondents.

Procedure. The first round of the study was conducted at the beginning of the Covid-19 pandemic. Data collection lasted three weeks, from March 22 to April 11, 2020, during the first lockdown in the Czech Republic. The second round was conducted after most restrictions had been (temporarily) lifted, between June 11 and July 4, 2021, when the pathogen threat substantially decreased. The priming consisted of two stories: (i) a story about people who became infected with Covid-19 and violated the quarantine (this focused on the threat of contagion and moral disgust and served as the experimental condition), and (ii) a story about the meltdown of a famous iceberg in Iceland (this concentrated on the threat posed by global warming and served as the control condition). To obtain balanced sample sizes for each priming condition, all even-numbered participants were in this first round assigned to experimental priming and all odd-numbered participants to the control priming condition. During the second round of data collection, all respondents were exposed to the same priming condition as they were in the first round.

At the beginning, respondents completed a sociodemographic inventory, inventory on long-term and current health, Personality Inventory, and Trait Anxiety Inventory. Responses provided for the initial set of inventories

were not expected to fluctuate because they aimed at assessment of traits and their order was therefore not randomized. After the initial part, we displayed the priming story. Priming was followed by a set of questionnaires in a randomized order (State Anxiety Inventory, Perceived Stress Scale, Three Domain Disgust Scale, Culpepper Disgust Image Set, Body Odor Disgust Scale, and Hygienic Behavior). Participants also completed the Social Phobia Inventory and Xenophobia Inventory. At the end of the survey, participants completed a priming-related memory test. The results of the State Anxiety Inventory, Perceived Stress Scale, Personality Inventory, Hygienic Behavior Inventory, Social Phobia Inventory and Xenophobia Inventory will be presented elsewhere.

Moreover, we monitored the epidemiologic situation in the Czech Republic and recorded it in a "Covid diary," which was updated daily based on information published on the website of the Ministry of Health and novinky.cz (a large online news provider in the Czech Republic). We recorded the number of new reported Covid-19 cases and deaths, as well as changes in local epidemiological restrictions. We started keeping the diary one month before launching the first round and ended one month after finishing the second round of the study. The "Covid diary" served as a control for the epidemiological situation mainly during data collection: we wanted to keep track of any significant changes in the epidemiological situation.

Priming stories. The priming story in the experimental condition aimed at evoking the threat of contagion (pathogen disgust) and violation of rules (moral disgust). The main characters, a married couple, go skiing in northern Italy at the beginning of the coronavirus pandemic and become sick with Covid-19 a few days after their return. Although the husband is hospitalized, his wife violates the quarantine and visits her neighbors, an elderly couple. Later, one of the neighbors falls ill, develops severe pneumonia, and has to be put on artificial ventilation. The story was based on a common route of coronavirus spread from returning travelers to the Czech Republic and it took into account the increased risks Covid-19 poses to older persons. For details, see the P1 in the Supplementary materials.

In the control priming story, a married couple travel to Faxaflói, Iceland, to cross the famous iceberg Okjökull only to discover that there is no snow and temperatures are relatively high. They wait for a few days, but the situation does not improve, so they decide to return home earlier. The wife visits her neighbors, an elderly couple, who tells her that the Okjökull is melting down. Moreover, the neighbors tell her that the owner of an inn where both couples had recently spent their holidays may soon have to close the business because of dramatic climate changes. For details, see the P2 in the Supplementary materials.

We developed the two stories to vary in the motif of threat while remaining comparable in terms of length, storyline, and the number of characters. Also, we informed respondents before they read the priming story that there would be a memory test at the end of the survey: in this way, we wanted them to focus on the stories and see which priming would affect the memory score more.

We validated the priming stories in a pilot study which was conducted at the beginning of March 2020 during the first lockdown, for which we recruited a total of 152 respondents (110 women and 42 men) aged at least 18 years old (women: $M_{age} = 38.9$, $SD = 13.3$, men: $M_{age} = 40.1$, $SD = 12.2$) (74 in experimental condition). We asked them to verbally rate the credibility of the assigned priming story ("How plausible was the story you read?", "How engaging was the story?", "Is there anything you would change to make the story more credible and impressive?", "Which emotions did the story elicit in you?"). Furthermore, the pilot study contained items on basic sociodemographic status, the Ten-Item Personality Inventory (TIPI)[24], the Trait Anxiety Inventory (TAI)[25], the Three Domain Disgust Scale (TDDS)[4], and a memory test, which assessed the memorability of our priming stories. We adapted the stories based on feedback from the respondents and made them more closely matched: we compared them sentence by sentence and adjusted them to match the corresponding sentence from the other story. For results of the pilot study, see S1 in the Supplementary materials.

Measures. The sets of questionnaires were completed online through the Qualtrics survey platform. To start with, we asked some basic sociodemographic questions (such as age, gender, and education) and questions about long-term and current health issues (e.g. "How often do you suffer from headaches?" or "Do you use any medication prescribed by your family doctor?"). The health score was obtained from 9 items covering the incidence of health issues such as headaches, colds, or fatigue. The respondents rated each item on a verbally anchored 8-point scale. Response options were: "I don't suffer from this issue at all" (scored as 0), "less than once a year", "once a year", "twice a year", "every three months", "once a month", "once a week", and "more often" (scored as 7). The final score could range from 0 to 63, with higher scores indicating a higher incidence of health issues. For a list of sociodemographic and health-related items, see S2 in the Supplementary materials. In the second round of the study, the sociodemographic questionnaire was considerably reduced. We focused on variables that may have changed and left aside variables such as height, weight, and occupation.

Trait anxiety. To assess trait anxiety, we used the 20-item Trait Anxiety Inventory (TAI), which explores stable anxiety traits[25]. It has been reported that anxiety traits affect disgust sensitivity[26]. Respondents were asked to assess how often they experienced feelings described in the questions. These self-reported questionnaires were rated on a 4-point scale. The possible replies for TAI were: "almost never" (scored as 1), "sometimes", "often", "almost always" (scored as 4). The scores for the test ranged between 20 and 80, with higher scores indicating higher anxiety levels.

Disgust measures. Disgust sensitivity was assessed by three different measures: (i) Three Domain Disgust Scale, (ii) Culpepper Disgust Image Set, and (iii) Body Odor Disgust Scale. A higher score in each measure indicated a higher level of disgust sensitivity.

The Three Domain Disgust Scale (TDDS)[4] is a self-report questionnaire with 21 items consisting of three subscales (pathogen, moral, and sexual disgust), with 7 items in each subscale. The items were rated on a 7-point scale ranging from 0 (not disgusting at all) to 6 (extremely disgusting). The total score could range between 0 and 126 and each subscale between 0 and 42.

The Culpepper Disgust Image Set (C-DIS)[27] contains 20 pathogen-salient and paired 20 pathogen-free images divided in four factors: hygiene issues, parasite/infection, food/environmental, and injury/viscera. The images were rated on a 7-point scale ranging from 0 (not at all disgusting) to 6 (extremely disgusting). Mean scores were computed for both groups of images. Finally, scores for images suggestive of pathogen-free conditions were subtracted from scores for the pathogen-salient images to obtain the final score.

The Body Odor Disgust Scale (BODS)[28] is a self-report questionnaire with 12 items focused on body odors eliciting disgust. It is divided in two subscales depending on whether the source of odor is external (e.g. "You are sitting next to a stranger and notice that his feet are very smelly.") or internal (e.g. "You are alone at home and notice that your feet are very smelly."). Participants rated each item on a 5-point scale ranging from 1 (not disgusting at all) to 5 (extremely disgusting). The score for each domain was calculated as a mean value. Final scores could range between 1 and 5.

Memory test. At the end of the set of the questionnaires, participants completed a 10-item memory test with multiple-choice questions (five possible answers) with one correct answer based on the previous priming story, e.g. "What was the name of the man in the opening story?" or "How many days did they spend on their holidays?". For the list of questions, see S3 in the Supplementary materials. The score could range between 0 and 10 points.

Covid-related items. After completing the memory test, respondents answered a question related to their Covid concerns ("Are you concerned about the coronavirus?") and a question about avoidance of human contact ("Are you avoiding travel, public places, or places with a higher concentration of people because of the coronavirus?"). The items were rated on a scale ranging from 0 to 100, with higher values indicating a higher level of concern/avoidance.

Statistical analyses. All statistical tests were performed using Jamovi v. 0.9.6.9 software (The jamovi project, 2021; https://www.jamovi.org). To assess possible relationships between the dependent variables (disgust scores [TDDS, C-DIS, BODS], and the memory test) and two independent variables, namely the priming condition and data collection point (in the spring of 2020 during the first lockdown or during temporary lifting of restrictions in summer 2021), we employed a repeated measure Analysis of Covariance (ANCOVA). In the follow-up analyses, we explored interindividual differences, including control for possible effects of sex, age, health status, and trait anxiety on the abovementioned dependent variables.

We excluded from the analysis incomplete questionnaires (with over one-fifth of each domain or questionnaire unanswered) and questionnaires with the same values across most items. Missing data in incomplete questionnaires (with less than a fifth of each domain) were supplemented by average scores for that domain. We also excluded respondents under 18 years of age and pregnant women because disgust sensitivity changes during pregnancy[29,30].

Results

The effect of priming on perceived disgust during a period of high pathogen threat. The statistical analysis included 761 respondents, 379 of whom (293 women, 86 men) were exposed to the experimental priming and 382 individuals (293 women, 89 men) to the control priming. Table 1 shows descriptive statistics

	Experimental priming (N = 379)				Control priming (N = 382)			
	Women (N = 293)		Men (N = 86)		Women (N = 293)		Men (N = 89)	
	M	SD	M	SD	M	SD	M	SD
Age	33.8	11.8	34.3	11.6	32.3	11.8	32.5	9.5
TDDS total	70.8	19.4	58.5	18.4	68.5	18.8	54.1	18.8
TDD-pathogen	23.8	7.6	21.1	7.4	23.1	7.4	19.0	7.0
TDD-moral	28.8	8.5	29.0	8.7	28.5	8.0	26.7	9.1
TDD-sexual	19.2	8.6	10.8	6.7	18.2	8.2	10.3	6.2
BODS-internal	2.9	0.9	2.8	0.8	3.0	0.9	2.6	0.9
BODS-external	4.0	0.7	3.8	0.7	4.0	0.7	3.6	0.9
C-DIS	3.8	0.8	3.7	0.9	3.9	0.8	3.8	0.8

Table 1. Descriptive statistics for measures from respondents collected during the period of high pathogen threat in experimental and control priming conditions.

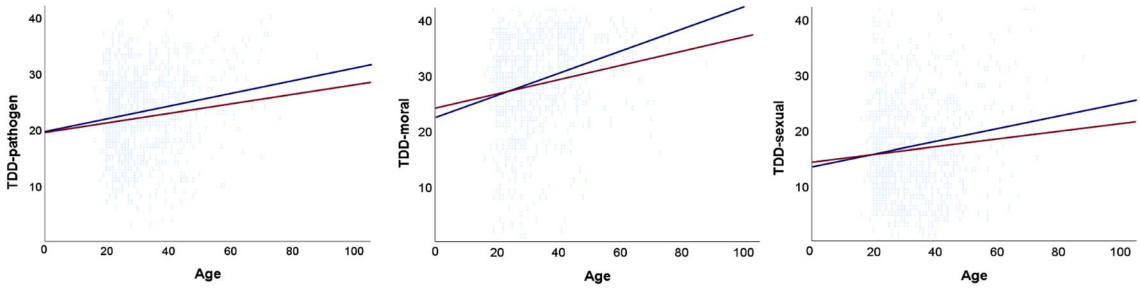

Figure 1. The association between respondents' age and pathogen, moral, and sexual disgust scores in the experimental priming condition (blue line) and control priming condition (red line) during the period of high pathogen threat.

separately for women and men for parameters, such as age and disgust measures, collected during the period of high pathogen threat.

First, we compared the age between the experimental and control priming group and found that respondents in the experimental priming group were significantly older: $t(759) = 1.85$, $p = 0.033$, $d = 0.134$. To account for this confounding variable, we added age as a covariate to further analyses (see Fig. 1). Although women scored significantly higher on most disgust measures, we did not find a significant interaction between the priming condition and sex on disgust measures (see Table S4 in the Supplementary materials). The ANCOVA revealed a strong effect of age on all TDDS subscales scores, BODS-internal scores, and C-DIS scores but during the period of high pathogen threat, the effect of priming on TDDS total scores was not significant (see Table 2).

In line with preregistration, we analyzed individual TDDS subscales separately, because we expected the effect of priming to be specific to pathogen disgust and moral disgust. We found no differences between experimental and control priming conditions in pathogen, moral, or sexual disgust. Moreover, the effect of priming was bordering on a formal level of statistical significance in C-DIS score but in the opposite than predicted direction: C-DIS score was higher in the control condition than in the experimental condition, although the effect size was not large. Finally, we found no significant effect of priming on either the BODS-internal or BODS-external score.

For the descriptive statistics of participants who dropped out after the first round of data collection, see S5 in the Supplementary materials.

Comparing BIS-related variables during the period of high and low pathogen threat. The statistical analysis included 224 respondents who completed the survey during both data collection rounds: 99 participants (80 women, 19 men) were exposed to the experimental priming and 125 participants (95 women, 30 men) to the control priming. Descriptive statistics for parameters age, health score, trait anxiety, disgust measures, memory test score and covid concerns and avoidance, are presented in Table 3.

Disgust measures. A repeated measure ANCOVA with age, health score, and TAI score as covariates and the priming condition as a between-subject factor found that during the period of high pathogen threat, respondents did not score significantly higher in TDDS total score, pathogen, or sexual disgust scores than during the period of low pathogen threat. Respondents did, however, score significantly higher on the moral disgust sub-

Variable		F	p	η^2_p
TDDS total score	Priming	2.68	0.102	0.004
	Age	**41.81**	**< 0.001**	**0.052**
TDD-pathogen	Priming	3.03	0.082	0.004
	Age	**18.10**	**< 0.001**	**0.023**
TDD-moral	Priming	0.76	0.382	0.001
	Age	**37.59**	**< 0.001**	**0.049**
TDD-sexual	Priming	1.44	0.231	0.002
	Age	**11.11**	**< 0.001**	**0.015**
BODS-internal	Priming	0.16	0.686	0.000
	Age	**5.35**	**0.021**	**0.007**
BODS-external	Priming	0.56	0.454	0.001
	Age	0.59	0.442	0.001
C-DIS	Priming	3.66	0.056	0.005
	Age	**6.36**	**0.012**	**0.009**

Table 2. Series of ANCOVA models testing the effect of priming and age on TDDS, BODS, and C-DIS during the period of high pathogen threat. Statistically significant associations are marked in bold.

	High pathogen threat (N = 224)								Low pathogen threat (N = 224)							
	Experimental priming (N = 99)				Control priming (N = 125)				Experimental priming (N = 99)				Control priming (N = 125)			
	Women (N = 80)		Men (N = 19)		Women (N = 95)		Men (N = 30)		Women (N = 80)		Men (N = 19)		Women (N = 95)		Men (N = 30)	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Age	32.4	11.8	33.9	14.2	31.1	10.2	31.2	11.3	33.6	11.8	35.1	14.1	32.4	10.2	32.3	11.3
Health score	28.7	8.4	19.7	9.04	27.2	9.3	23.2	9.4	27.6	8.3	19.2	8.2	25.9	9.2	21.8	10.0
TAI	47.3	11.8	41.1	10.9	46.4	11.7	40.8	9.6	46.6	10.9	41.4	9.1	45.9	11.5	40.3	10.9
TDDS total	70.6	18.0	56.5	15.9	65.4	19.1	52.0	17.9	63.5	21.5	53.8	20.4	61.5	19.2	50.2	19.3
TDD-pathogen	23.6	7.1	19.6	5.7	21.5	7.9	17.9	6.4	23.4	8.3	19.1	6.2	22.0	8.7	18.9	7.6
TDD-moral	28.0	9.7	26.9	11.0	26.4	10.1	25.5	11.6	22.3	11.4	24.7	13.3	22.3	10.9	21.9	11.7
TDD-sexual	19.1	8.2	10.0	4.7	17.5	8.2	8.6	5.3	17.4	8.6	10.0	5.7	17.2	7.6	9.4	5.8
BODS-internal	3.0	0.9	2.7	0.7	2.8	0.9	2.7	0.9	2.8	0.9	2.6	0.8	2.6	0.9	2.5	0.8
BODS-external	4.1	0.7	3.6	0.8	3.8	0.8	3.6	1.0	4.0	0.7	3.6	0.9	3.8	0.8	3.5	0.9
C-DIS	3.9	0.7	3.5	0.8	3.8	0.9	3.3	0.8	4.0	0.8	3.7	0.6	3.8	1.0	3.5	0.9
Memory score	7.2	1.8	6.1	1.8	7.4	1.8	7.0	1.8	7.0	1.9	6.2	1.7	7.3	1.9	7.1	1.4
Covid concerns	63.6	27.0	63.5	26.9	61.1	28.1	46.7	27.7	50.9	25.6	43.9	29.9	45.5	26.2	49.4	29.3
Covid avoidance	84.4	21.7	81.2	24.0	81.0	25.0	83.7	27.9	50.2	31.1	54.3	32.2	48.1	31.9	42.3	30.5

Table 3. Descriptive statistics for measures from respondents participating during both the high and low pathogen threat period.

scale during the period of high pathogen threat. We found no statistically significant differences between the BODS subdomains scores and C-DIS scores between the two data collection points. Moreover, we found no significant interaction between the data collection point and the priming conditions. Age was positively associated with TDDS total score, moral disgust score, and C-DIS score. Furthermore, trait anxiety scores significantly positively affected TDDS total scores, pathogen and sexual disgust scores, BODS-internal scores, and C-DIS scores. For more detailed results, see Table 4. To investigate significant interactions, we further analyzed the association between TAI and BODS–internal separately for the period of high and low pathogen threat. We found a slightly stronger effect of TAI on BODS-internal during the period of low pathogen threat ($r = 0.291$, $p = < 0.001$) than during the period of high pathogen threat ($r = 0.156$, $p = 0.020$), but the effect did not reach the threshold of statistical significance ($z = 1.497$, $p = 0.067$). A repeated measure ANCOVA with sex as an additional covariate can be found in the Supplementary materials (see Table S6).

We found no statistically significant differences between memory scores achieved during the periods of high vs. low pathogen threat (see Table S3 in the Supplementary materials). Our results also showed no differences in trait variables between the two time points (see Table S7 in the Supplementary materials).

Covid concerns. A repeated measure ANCOVA with age, health score, and TAI score as covariates and the priming condition as a between-subject factor found that during the period of high pathogen threat, respondents reported a statistically significantly higher level of avoidance of other people than during the period of low pathogen threat. We did not, however, find the same effect in relation to Covid concerns. TAI scores were positively associated with Covid concerns but not with Covid avoidance. Moreover, we found a significant interaction between Covid concerns and age, but the interaction differed between the rounds of data collection. For more detailed results, see Table 5. To investigate this significant interaction, we have analyzed the association between Covid concerns and age separately for the period of high and low pathogen threat. We found a stronger effect ($z = 1.615$, $p = 0.05$) of age on Covid concerns during the period of low pathogen threat ($r = -0.117$, $p = 0.086$) than during the period of high pathogen threat ($r = 0.038$, $p = 0.572$).

Moreover, we found positive associations between Covid concerns and majority of the disgust measures during the high pathogen threat period but not during the low pathogen threat period. Covid avoidance was negatively associated with BODS-internal and positively associated with TDDS total score during the period of high pathogen threat (see Table S8 in the Supplementary materials).

Association between BIS-related variables and TAI and health scores. As predicted in the preregistration, we found positive associations between scores of trait anxiety and disgust measures during the high and low pathogen threat periods (see Table 6). These results indicate that respondents who scored higher on trait anxiety also scored higher on disgust. Similarly, we found positive associations between health scores and most of the disgust measures at both data collection points (see Table 6). Respondents who reported more health issues also scored higher on disgust.

Association between age and pathogen and moral disgust. We found a significant positive correlation between age and the moral disgust score ($r = 0.165$, $p = 0.014$) but not pathogen disgust score ($r = 0.128$, $p = 0.055$) or sexual disgust score ($r = 0.068$, $p = 0.314$) during the period of high pathogen threat. We also found

Parameter name		F	p	η^2_p
TDDS total score	Period	2.995	0.085	0.013
	Priming	1.770	0.185	0.008
	Age	10.260	**0.002**	0.045
	TAI	9.140	**0.003**	0.040
	Health score	1.510	0.221	0.007
	Period * priming	2.787	0.096	0.013
	Period * age	0.064	0.801	0.000
	Period * TAI	0.068	0.794	0.000
	Period * health score	0.544	0.462	0.002
TDD-pathogen	Period	0.188	0.665	0.001
	Priming	2.400	0.123	0.011
	Age	1.830	0.178	0.008
	TAI	9.500	**0.002**	0.042
	Health score	1.290	0.258	0.006
	Period * priming	0.967	0.326	0.004
	Period * age	5.575	**0.019**	0.025
	Period * TAI	0.172	0.678	0.001
	Period * health score	0.798	0.373	0.004
TDD-moral	Period	6.667	**0.010**	0.030
	Priming	0.255	0.614	0.001
	Age	13.276	< **0.001**	0.057
	TAI	1.330	0.250	0.006
	Health score	0.286	0.593	0.001
	Period * priming	1.087	0.298	0.005
	Period * age	4.292	**0.039**	0.019
	Period * TAI	0.082	0.775	0.000
	Period * health score	0.218	0.641	0.001
TDD-sexual	Period	1.262	0.262	0.006
	Priming	0.938	0.334	0.004
	Age	2.148	0.144	0.010
	TAI	6.748	**0.010**	0.030
	Health score	1.162	0.282	0.005
	Period * priming	1.262	0.262	0.006
	Period * age	0.008	0.931	0.000
	Period * TAI	0.457	0.500	0.002
	Period * health score	0.072	0.789	0.000
BODS-internal	Period	2.495	0.116	0.011
	Priming	2.160	0.143	0.010
	Age	1.630	0.202	0.007
	TAI	8.160	**0.005**	0.036
	Health score	1.400	0.237	0.006
	Period * priming	0.061	0.804	0.000
	Period * age	3.088	0.080	0.014
	Period * TAI	5.581	**0.019**	0.025
	Period * health score	0.038	0.845	0.000
BODS-external	Period	0.443	0.506	0.002
	Priming	3.100	0.080	0.014
	Age	1.510	0.221	0.007
	TAI	3.280	0.072	0.015
	Health score	1.820	0.179	0.008
	Period * priming	0.224	0.637	0.001
	Period * age	1.865	0.173	0.008
	Period * TAI	3.132	0.078	0.014
	Period * health score	0.313	0.576	0.001

Continued

Parameter name		F	p	η^2_p
C-DIS	Period	0.374	0.541	0.002
	Priming	1.430	0.233	0.007
	Age	5.280	**0.023**	0.026
	TAI	6.230	**0.013**	0.030
	Health score	1.170	0.280	0.006
	Period * priming	0.049	0.825	0.000
	Period * age	0.145	0.704	0.001
	Period * TAI	0.075	0.784	0.000
	Period * health score	0.644	0.423	0.003

Table 4. Series of the repeated measure ANCOVAs testing the effect of the period (high and low pathogen threat) and the priming condition on TDDS, BODS, and C-DIS scores controlling for age, health score and TAI. Df is 1 for all variables and 219 for residuals. Statistically significant associations are marked in bold.

Parameter name		F	p	η^2_p
Covid concerns	Period	0.073	0.787	0.000
	Priming	1.216	0.271	0.006
	Age	0.025	0.874	0.000
	TAI	6.814	**0.010**	0.032
	Health score	0.001	0.970	0.000
	Period * priming	0.009	0.924	0.000
	Period * age	8.045	**0.005**	0.037
	Period * TAI	0.605	0.437	0.003
	Period * health score	0.717	0.398	0.003
Covid avoidance	Period	16.459	**< 0.001**	0.075
	Priming	0.477	0.491	0.002
	Age	0.025	0.875	0.000
	TAI	1.910	0.168	0.009
	Health score	2.704	0.102	0.013
	Period * priming	1.019	0.314	0.005
	Period * age	0.558	0.456	0.003
	Period * TAI	1.855	0.175	0.009
	Period * health score	0.498	0.481	0.002

Table 5. Series of the repeated measure ANCOVAs testing the effect of the period (high and low pathogen threat) and the priming condition on Covid concerns and avoidance controlling for age, health score and TAI. Df is 1 for all variables and 209 for residuals. Statistically significant associations are marked in bold.

	High pathogen threat				Low pathogen threat			
	TAI score		Health score		TAI score		Health score	
	r	p	r	p	r	p	r	p
TDDS total score	0.244	< 0.001	0.172	0.005	0.235	< 0.001	0.191	0.002
TDD-pathogen	0.244	< 0.001	0.164	0.007	0.228	< 0.001	0.205	0.001
TDD-moral	0.109	0.052	0.073	0.138	0.087	0.097	0.032	0.319
TDD-sexual	0.204	0.001	0.154	0.011	0.227	< 0.001	0.214	< 0.001
BODS-internal	0.171	0.005	0.151	0.012	0.295	< 0.001	0.199	0.001
BODS-external	0.132	0.025	0.151	0.012	0.171	0.005	0.175	0.004
C-DIS	0.116	0.045	0.009	0.446	0.112	0.052	0.077	0.133

Table 6. Partial Pearson's correlation between disgust measures, TAI, and health scores controlled for age.

a significant positive association between age and moral disgust score ($r = 0.252$, $p = < 0.001$) but not pathogen disgust score ($r = -0.015$, $p = 0.826$) and sexual disgust score ($r = 0.063$, $p = 0.346$) during the period of low pathogen threat.

To further explore the effect of age on the pathogen and moral disgust scores, we split respondents in four age categories and repeated the analysis (a repeated measure ANCOVA) with age groups as a between-subject factor. We found a statistically significant interaction between data collection point (high vs. low pathogen threat) and age group in pathogen disgust score, $F_{3,220} = 4.06$, $p = 0.008$, $\eta^2_p = 0.052$, but not in moral disgust score, $F_{3,220} = 1.18$, $p = 0.316$, $\eta^2_p = 0.016$. Tukey's HSD Test for multiple comparisons showed that the effect was strongly dependent on the group of 50–69 years old respondents regarding pathogen disgust because this was the only age category that differed between the data collection points ($p_{tukey} = 0.05$); see Fig. 2.

Discussion

The main aim of the current study was to test whether the perception of high pathogen threat caused by the outbreak of the Covid-19 pandemic led to an increase in disgust sensitivity (especially pathogen and moral disgust). The perception of threat was induced by (i) a priming story and (ii) testing during a period of high pathogen threat (the beginning of the pandemic in the Czech Republic, the first lockdown) as compared to a period of low pathogen threat (14 months later, at a time when the Covid-19 incidence was relatively low). In contrast to our predictions, the results did not show heightened disgust in response to the experimental priming condition compared to the control priming condition at either data collection point. During the period of high pathogen threat respondents scored significantly higher on moral disgust but not on pathogen disgust than during the period of lower pathogen threat. Moreover, the effect was modulated by the age of respondents, being the highest among senior respondents.

Our findings regarding elevated moral disgust during periods of high pathogen threat are in line with a previous study which showed that individuals from nations with a higher pathogen burden tend to follow group norms more strictly[31] and moral judging increases after exposure to disgusting odors or video clips[22]. Other studies, however, failed to replicate these results and the overall picture is thus rather ambivalent[32]. In our study, we also found a significant interaction between the data collection point and the age of respondents, with the highest moral disgust in the oldest age category. This might be due to respondents' awareness of Covid-19 posing increased risk of serious illness in older persons[33]. Therefore, they may perceive any violation of rules as more disgusting.

Our finding of no difference in pathogen disgust between the periods of low and high pathogen threat conflicts with the results of several other studies which did report higher scores on self-reported disgust scale[14] or higher disgust sensitivity using a naturalistic measure[12] during a period of high pathogen threat. In the latter study, however, the difference in the TDDS pathogen domain between groups assessed before and during a pandemic "wave" was also not formally statistically significant. The disgust measures based on statements were originally designed for assessing interindividual variation, so it is possible that they are ill-suited for detection of intraindividual variation, such as one may find during periods of immune vulnerability[34]. The absence of a difference in pathogen disgust between the two data collection points (during a lockdown and after restrictions were lifted) may be due to the use of the TDDS questionnaire, which seems to be less sensitive to environment-related fluctuations in pathogen avoidance. Interestingly, we also failed to find the difference using the C-DIS, which includes images of disease cues and is considered to be a more sensitive measure of variation in pathogen disgust.

It should be noted that the studies reviewed above compared two different groups of respondents at different time points (e.g. before and during pandemic). It is thus possible that in our study, pathogen disgust measured during a period of low pathogen threat (after most restrictions were lifted) remained elevated and was as high as at the beginning of the pandemic. It is perhaps because the threat was perceived as not being quite over yet. Nevertheless, the difference in incidence of Covid-19 infections (44 per 100,000 persons and 25 per 100,000 persons) between the two periods clearly shows that our decision to treat the two time points as periods high and low pathogen threat was justified (incidence is almost doubled), although the months that followed showed that case numbers could rise to values[35] we could not anticipate when we designed the study in March 2020, at the outset of the pandemic which lasted globally for over two years. Nevertheless, one may argue that the two periods did not differ in overall pathogen threat as we expected, because we took into account only the incidence

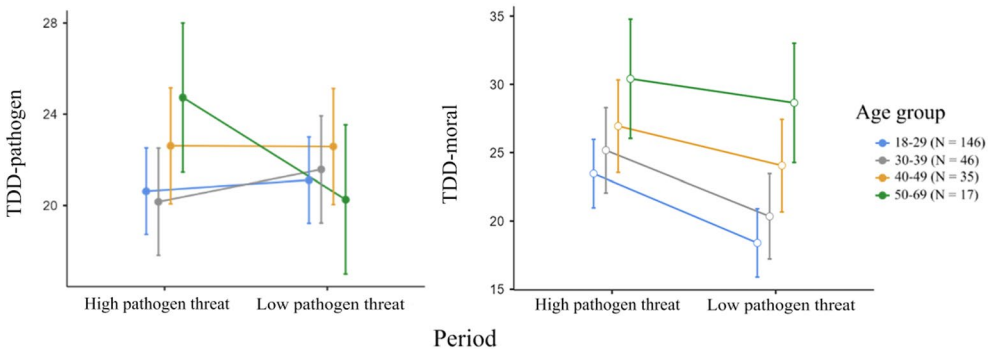

Figure 2. Comparison of age groups in pathogen and moral disgust score; error bars indicate 95% CI.

of Covid-19 cases but not of other infectious agents. Data from the National Institute of Public Health in Czech Republic (https://szu.cz/publikace/data/akutni-respiracni-infekce-chripka/) show that at the beginning of the Covid pandemic, influenza as a viral respiratory tract infection causing morbidity and mortality worldwide was still active, although its incidence was slowly decreasing. In early April 2020, the incidence of influenza was rather low and hotspots local, while Covid-19 started to be more prominent. The entire season of 2020/2021 was significantly influenced by Covid-19 and associated restrictions, which caused a milder course of the seasonal influenza epidemic. Overall data show that the spread of influenza was during the Covid-19 pandemic drastically reduced compared to previous years[36]. Such massive reduction in case numbers has been attributed primarily to mask wearing, which was in the Czech Republic obligatory during the period of high pathogen threat, while lower levels of contact among people also played a significant role. Importantly, objective measures of high pathogen threat (e.g. incidence of new cases) need not have directly translated into subjectively perceived threat level and consequently to higher levels of disgust. It is, for instance, possible that during the lockdown, people were exposed to markers of infection—such as coughing—to a smaller extent because most of the time they stayed at home. Moreover, a substantial percentage of people who become infected with Covid-19 remain asymptomatic and many transmission routes do not provide sufficient cues[37]. This might help explain why we did not find any elevation of pathogen disgust. Nevertheless, we do not think this can entirely explain the null finding because the respondents did report being more concerned about Covid-19 during the period of high pathogen threat and they also reported that they avoid public spaces and travel.

The lack of difference in pathogen disgust between the periods of high and low pathogen threat might be also caused by the selection bias. Specifically, respondents who showed elevated disgust levels may have been more likely to participate in the second round of data collection. But this explanation seems unlikely. We compared individuals who took part only in the first round and those who participated in both rounds and found that respondents who took part in both rounds of data collection scored lower on pathogen and moral disgust than those who took part only in the first round. This is the opposite of what one would expect in a case of selection bias for null findings.

We found no effect of the priming condition on disgust sensitivity, which was expected in the preregistration. We predicted that an elevation of disgust levels during the period of high pathogen threat would preclude further efficiency of the experimental priming. During the period of low pathogen threat, on the other hand, we expected to see an increase in disgust sensitivity after exposure to the experimental priming. The stories were carefully designed during the first wave of the Covid-19 pandemic in spring 2020 and provisionally validated based on a data from the pilot study. The use of vignettes is a common tool used in psychological research[38] and vignettes are also widely employed in a research on sexual and moral disgust[17–19]. Nevertheless, one might argue that pathogen disgust was selected primarily to respond to perceptual cues of infection (e.g. the smell of wounds), while indirect stimuli—such as the story we used—need not be an efficient disgust trigger. On the other hand, there is robust evidence to the effect that affective states, including disgust, can be easily induced by symbolic communication either in spoken or written form[39]. Furthermore, disgusting stories boost item recall and recognition[40], although our results did not show any difference in the memory test results between the experimental and control priming story.

We also found that older respondents showed overall higher pathogen and moral disgust than younger respondents did at both data collection points, which is partly in line with our preregistered hypotheses and our pilot study. We assumed that older persons are more vulnerable to diseases because the functionality of their physiological immune system is declining[41]. Their disgust sensitivity and functionality of the BIS should therefore be elevated to compensate for this possible deficiency[42]. This assumption finds support in a recent study which found that germ aversion increases with age[15]. Our results also support the notion of BIS functioning as a compensatory mechanism because the increase in pathogen disgust at a time of high pathogen threat was restricted to the oldest age group (50–69 years). On the other hand, it has also been predicted that disgust sensitivity should decline with declining reproductive potential (i.e. with age). For example, Curtis et al.[6] found that younger participants were more disgusted by pathogen-salient pictures than older participants were. Several other studies similarly reported a negative correlation between age and disgust levels[7,43,44], but it should be noted that most of those studies did not involve participants over fifty, when further changes may occur (as shown by our data).

Our results also did not show any significant changes in trait anxiety and general health status between the two data collection points, which is in line with the preregistered hypotheses. These variables are considered stable. They can, however, influence the disgust scores: for instance, scores from trait anxiety and scores from the health status questionnaire were positively correlated with most of the disgust scores. These results are thus in line with the results of other studies, which also found a positive association between disgust sensitivity, health, and general anxiety scores[45,46].

Limitations. The current study relied on self-report measures. This can be considered a limitation because respondents need not be aware of changes in their disgust sensitivity, but they may still occur. Nevertheless, respondents also reported a smaller tendency to meet other people and slightly higher levels of concern about the coronavirus during the high-risk period. This indicates avoidance of the obvious routes by which Covid-19 spreads, but it may not be directly linked to pathogen disgust sensitivity.

Inspection of the age distribution of our sample shows that a rather small part of participants was over fifty years of age and majority of the sample was composed of young adults. Nevertheless, most of the participants samples in previous studies consist of young people. Clearly, more studies involving older participants are needed to better understand the age-dependent dynamics of disgust sensitivity.

Although we also carefully designed and provisionally validated the priming vignettes, their content may have been perceived as less relevant during the period of low pathogen threat, in summer 2021, which may have

limited their efficiency. It points to the variable dynamics of the Covid-19 pandemic and shows that scholars should differentiate between the individual waves of the pandemic[47,48].

Conclusions

Our findings based on a within-subject study show heightened moral disgust, but not increased pathogen disgust, during the first outbreak of the Covid-19 pandemic in the Czech Republic. Moreover, respondents' age positively correlated with both pathogen and moral disgust, supporting a hypothesis according to which the BIS may compensate for age-related decline in the functionality of the physiological immune system, which can come to the fore especially during a period of increased pathogen threat. Our study indicates that variations in pathogen disgust sensitivity—at least as measured by the TDDS—may depend more on age or trait anxiety as more stable characteristics and less on the relatively fast changing situation regarding current threats to health.

Our study also highlights the importance of differentiating between the objective level of threat and subjectively perceived threat. Finally, the findings indicate that the cognitive and perceptual components of BIS need not be always aligned: during the lockdown, the level of stimulation by pathogen cues (the perceptual component) might have been low, while concerns about infection (the cognitive component) were elevated. Future studies should therefore consider using a combination of behavioral tests and self-reports to capture various components of the BIS.

Data availability

The data associated with this research are available at (https://osf.io/sy6wn/).

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Author contributions

Conceptualization: D.S., Š.K., J.T.F., J.Ha. Data Curation: D.S., Š.K., J.Hl. Formal Analysis: D.S. Funding Acquisition: D.S., Š.K., J.Ha. Investigation: D.S., Š.K., J.Hl. Methodology: D.S., Š.K., J.T.F., J.Ha. Project Administration: D.S., Š.K., J.Hl. Supervision: Š.K., J.T.F., J.Ha. Validation: D.S., Š.K., J.T.F., J.Hl., J.Ha. Visualization: D.S., Š.K. Writing—Original Draft Preparation: D.S., J.Ha. Writing—Review & Editing: D.S., Š.K., J.T.F., J.Hl, J.Ha.

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The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to D.S.

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This is to confirm that Mgr. Dagmar Schwambergová significantly contributed to the following publication:

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She contributed to the conceptualisation, investigation, methodology, data analysis, visualisation, manuscript writing and subsequent editing and revisions.

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

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doc. Mgr. Jan Havlíček, Ph.D.
supervisor

Chapter 9

IMMUNOACTIVATION AFFECTS PERCEIVED BODY ODOR AND FACIAL BUT NOT VOCAL ATTRACTIVENESS

Evolutionary Psychology

Immunoactivation affects perceived body odor and facial but not vocal attractiveness

Journal:	*Evolutionary Psychology*
Manuscript ID	Draft
Manuscript Type:	Original Research Article
Keywords:	perception, body odour, voice, face, health status
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 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 and vocal 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

Dagmar Schwambergová[1]*, Žaneta Pátková[2], Jitka Třebická Fialová[1], Vít Třebický[3], David Stella[4], and Jan Havlíček[1]

[1] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic

[2] Department of Philosophy and History of Science, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic

[3] Faculty of Physical Education and Sport, Charles University, José Martího 31, 162 52, Prague, Czech Republic

[4] Global Change Research Institute CAS, Bělidla 986/4a, 603 00, Brno, Czech Republic

* Corresponding author:

Tel.: +420 221951853, fax: +420 221951125

E-mail: schwambd@natur.cuni.cz

Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic

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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 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 and 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 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 predicted that these characteristics will be rated as less attractive and less healthy after vaccination. Moreover, we anticipated that the levels of C-reactive protein (CRP), viewed as a marker of inflammation, would be higher after vaccination compared to the basal pre-vaccination levels.

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 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 × 7cm; DM Ebelin), 100% cotton white T-shirt (Adler Malfini Heavy), a non-perfumed soap (Balea ultrasensitive), and surgical tape (Omnisilk 2.5cm x 9.2m). 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 × 24mm CMOS sensor, a 35mm film equivalent) DSLR camera Nikon D610, with a Nikon AF-S Nikkor 85mm F1.8 AF-S G lens. Exposure was manually set to ISO 100 with shutter speed of 1/125s 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 175cm 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 50cm 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 125cm 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. Distance between the target and the camera (sensor plain marked ϕ) was verified with a digital laser rangefinder (Bosch PLR 15). This setting of camera distance, focal length, and sensor size gave a 35 × 53cm 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 × 53cm) 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 2nm, 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 40cm 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, and fundamental frequency (F0), which is related to voice pitch.

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 125cm (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

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 (ρ = 0.437, p = 0.048, 95% CI [-0.015, 0.740]) and a comparable but not statistically significant correlation for attractiveness (ρ = 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 (ρ = 0.883; p < 0.001, 95% CI [0.688, 0.960]) and after vaccination (ρ = 0.921; p < 0.001, 95% CI [0.780, 0.970]). Odor intensity rating negatively and statistically significantly correlated with both attractiveness (pre-vaccination: ρ = -0.827, p < 0.001, 95% CI [-0.937, -0.570]; post-vaccination: ρ = -0.520, p = 0.02, 95% CI [-0.789, -0.080]) and healthiness (pre-vaccination: ρ = -0.686, p < 0.001, 95% CI [-0.875, -0.320]; post-vaccination: ρ = -0.538, p = 0.016, 95% CI [-0.799, -0.110]). Because the correlation between attractiveness and healthiness reached the predefined threshold of ρ ≥ 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 (ρ = 0.930, p < 0.001, 95% CI [0.802, 0.980]) and healthiness (ρ = 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 (ρ = 0.706, p < 0.001, 95% CI [0.348, 0.880]) and post-vaccination condition (ρ = 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 (ρ = 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 (ρ = 0.317, p = 0.162, 95% CI [-0.144, 0.670]) but did find it in the post-vaccination condition (ρ = 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 (ρ = -0.066, p = 0.775, 95% CI [-0.484, 0.380]) or vocal attractiveness (ρ = 0.342, p = 0.129, 95% CI [-0.118, 0.680]), nor did it correlate with post-vaccination facial (ρ = -0.118, p = 0.609, 95% CI [-0.524, 0.330]) or vocal attractiveness (ρ = -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 and less healthy 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 association with 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.

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.*

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

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), and F0 (R^2_C = 0.245, R^2_M = 0.048) 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

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.

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 vocal characteristics (R^2_C = 0.446, R^2_M = 0.048). Higher CPPs and lower F0 thus predicted a target's voice being rated as more attractive (for details, see Table 7).

Characteristic	Parameters	F	B	95% CI (LL, UL)	df	t	SE	p
Vocal attractiveness	CPPs	7.72	0.167	0.049, 0.285	539.9	2.778	0.06	0.006
	HNR	3.46	-0.089	-0.184, 0.005	1422.5	-1.86	0.048	0.063
	F0	1.36	-0.011	-0.03, 0.008	287.3	-1.17	-0.011	0.028

Table 7: Relationships between acoustic measures (CPPs, HNR and F0) and perceived vocal attractiveness. For target ID: random components variance = 0.999, SD = 0.999, ICC = 0.372; 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, β = 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, β = -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, β = 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, β = 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, β = -0.007, 95% CI [-0.130, 0.115], p = 0.904, R^2 = 7.82e-4).

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.

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 F0 was negatively associated with attractiveness ratings (e.g., Hodges-Simeon, Gaulin & Puts, 2010; Skrinda et al., 2014), while 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. 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, 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). It should be noted that the most distinctive changes in body odor, face, or voice may take place shortly after vaccine administration – as seen in studies using LPS (Olsson et al., 2014; Henderson et al., 2017). The choice of sampling two weeks after vaccination might thus be appropriate for assessing the increase in antibody levels but not for assessing 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 subtle 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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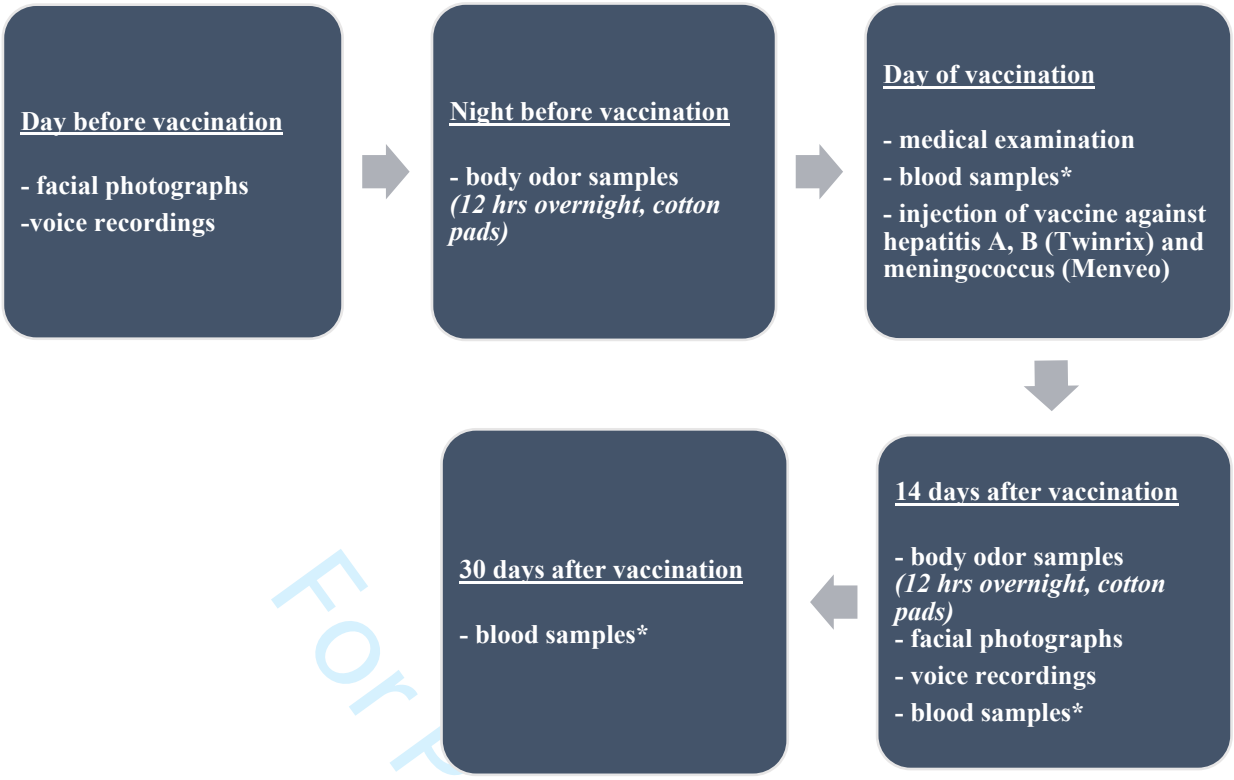

Fig. 1: Study schedule step by step. Approx. 48 hours before body odor collection, targets refrained from spicy and aromatic foods, alcohol, and increased physical activity. *Blood samples were collected to assess levels of specific antibodies, steroid hormones, and CRP.

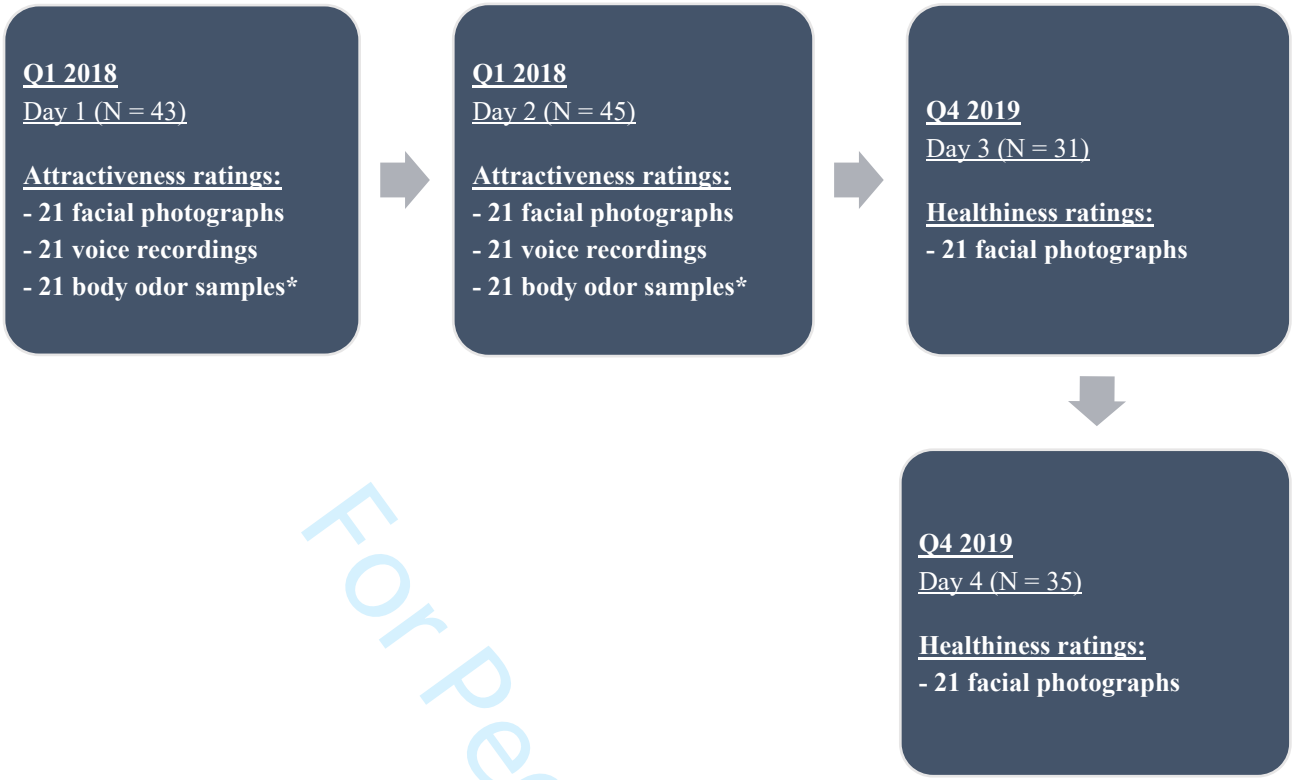

Fig. 2: Schedule of the rating procedure. All stimuli were rated on a 7-point scale for attractiveness (*body odor samples were rated for attractiveness, intensity, and healthiness at once) and healthiness. Stimuli from targets were presented to a rater on a given day either in the pre- or the post-vaccination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

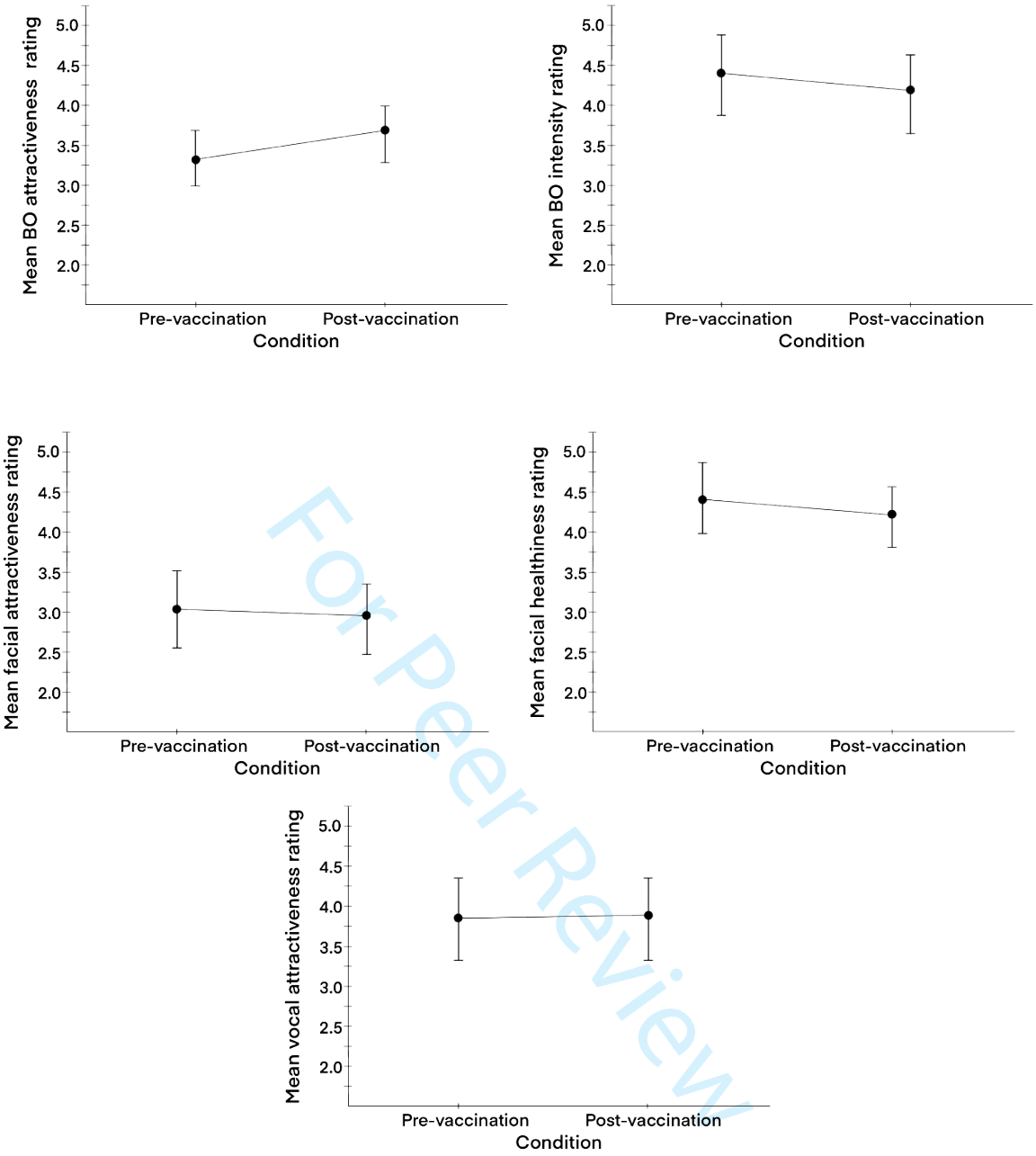

Fig. 3: Attractiveness (A) and intensity (B) of body odor samples, attractiveness (C) and healthiness (D) of faces and attractiveness of voices (E) depending on the condition (pre- vs. post-vaccination). Error bars show 95% confidence intervals.

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

				95% Confidence Interval				
Predictors	**F**	**β**	**SE**	**Lower**	**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

Statement of contribution

This is to confirm that Mgr. Dagmar Schwambergová significantly contributed to the following publication:

Schwambergová., D., Pátková, Ž., Třebická Fialová, J., Třebický, V., Stella, D., & Havlíček, J. (submitted). Immunoactivation affects perceived body odor and facial but not vocal attractiveness. *Evolutionary Psychology.*

She contributed to the conceptualisation, investigation, methodology, data analysis, visualisation, manuscript writing and subsequent editing and revisions.

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

_____

doc. Mgr. Jan Havlíček, Ph.D.
supervisor

Chapter 10

OLFACTION-MEDIATED PATHOGEN AVOIDANCE IN MAMMALS

Olfaction-Mediated Pathogen Avoidance in Mammals

Dagmar Schwambergová, Jitka Třebická Fialová, Jan Havlíček

Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Corresponding author: Dagmar Schwambergová, schwambd@natur.cuni.cz

Abstract

Avoidance of pathogens is crucial for one's survival. The mechanisms responsible for pathogen avoidance are known as the 'behavioural immune system', which consists of various perceptual, affective, cognitive, and executive processes. In this chapter, we review current knowledge on pathogen avoidance in mammals that is mediated by odour cues. We start with an overview of changes in the body odour caused by pathogens. Then we investigate current evidence regarding the avoidance of odours of infected individuals with emphasis on rodents and humans, and discuss effect of multimodal integration on avoidance behaviour. We review possible physiological mechanisms of pathogen avoidance, such as a decrease in pain sensitivity and hormonal involvement, including oxytocin, vasopressin, cortisol, and the sex hormones. Finally, we discuss ontogenetic development of pathogen avoidance. In conclusion, we outline some directions for future studies and point to the need to study a broader range of species.

Keywords: Behavioural immune system, Disgust, Immunity, Infection, Mammals, Odour.

The list of the taxons

anopheles mosquito (*Anopheles gambiae*)

Balantidium coli

banded mongoose *(Mungos mungo)*

brown rat (*Rattus norvegicus*)

Eimeria vermiformis

gonococcus *(Neisseria gonorrhoeae)*

Heligmosomoides polygyrus

Western European house mouse (*Mus domesticus*)

Eastern European house mouse (*Mus musculus*)

house mouse louse (*Polyplax serrata*)

Hymenolepis diminuta

common chimpanzee *(Pan troglodytes)*

mandrill *(Mandrillus sphinx)*

meadow vole *(Microtus pennsylvanicus)*

Mycobacterium mungi

Plasmodium falciparum

prairie vole *(Microtus ochrogaster)*

Salmonella enterica

Trichinella spiralis

Vibrio cholerae

1 Introduction

One of the key components to organisms' survival is pathogen avoidance. It is especially important in social species with high population densities, because such conditions sharply increase the risk of pathogen transmission (Altizer et al. 2003). The physiological immune system (PIS) and structural barriers, such as the skin and mucosae, are the body's main defence mechanism against pathogens. PIS plays a fundamental role in the recognition of structures of own versus foreign origin and in the activation of an adequate response. In vertebrates, PIS consists of innate and adaptive subsystems, which differ in their specificity as well as speed. Innate PIS is a nonspecific defence mechanism; as such, it is capable of providing an immediate response to pathogens. Adaptive PIS, on the other hand, is highly specific, but it may take days or even weeks to develop a response. Importantly, adaptive immunity develops "memory", which can considerably accelerate the response after repeated exposure to a specific pathogen. It should be noted that in most infections, the innate and adaptive PIS operate in concert, often forming a complex interconnected network (Parkin and Cohen 2001). But the system is energetically costly because recognition of a pathogen can trigger a cascade of metabolically demanding immune reactions (Sheldon and Verhulst 1996). The energy used to generate an immune response might be instead used for other purposes, such as reproduction or securing of food sources. Moreover, a physiological immune response often has a negative impact on the organism's functioning. Many symptoms of infection, including fever or fatigue, are not caused directly by pathogens: they are part of the immune system's response to pathogens. PIS should therefore be activated only in the presence of a relevant and actual threat. Moreover, given that PIS is activated only after the organism is actually infected, what should primarily be favoured is avoidance of potential sources of pathogens. It has been proposed that this is the role of the behavioural immune system (BIS), which thus serves as the first line of defence (Schaller and Duncan 2016).

This system contains various psychological mechanisms which help organisms avoid contagion from conspecifics, other animals, and inanimate objects (Schaller and Park 2011). More specifically, the BIS consists of complex processes, including perception and various affective, cognitive, and executive components. The detection of potentially threatening stimuli is based on visual, olfactory, gustatory, acoustic, and tactile cues, which may activate a negative affective response (in humans described as 'disgust'). An interplay of these components may then lead to avoidant behaviour.

Operation of the BIS can be conceived of in terms of the error management theory, which predicts that a system should favour less energy-demanding errors (Johnson et al. 2013). In other words, the theory claims that there is a higher susceptibility to false positive errors in order to reduce the probability of false negatives. The BIS thus operates analogously to a smoke detector, which is designed to be highly sensitive: it is triggered at the slightest hint of smoke, which can lead to frequent false positives (Nesse 2005, Schaller and Park 2011). On the other hand, this sensitivity is balanced by a drastic reduction of false negative errors that could lead to activation of the energy-demanding reactions of PIS or have other negative, even fatal, consequences. As a result, the BIS often evaluates some cues as potentially dangerous and triggers an

avoidance response even where there is no real risk. Moreover, unlike other threats, pathogens usually cannot be directly detected by our senses due to their size. Their activity can be discerned based on indirect cues, such as the smell of rotten food. This is another factor which contributes to the high alertness of the BIS.

The presence of a pathogen threat can alter the behaviour of individuals before they become infected. The BIS has been extensively studied in humans (Schaller and Park 2011), but various studies conducted on nonhuman mammals, such as rodents and primates, indicate that various mechanisms belonging to this system are shared (Curtis 2004, Kavaliers and Choleris 2018). This may also apply to disgust, which used to be thought to be unique to humans (Rozin et al. 2015), but recent studies conducted on great apes suggest that disgust is a part of pathogen avoidance in other species as well. For instance, a large survey among fieldwork researchers who work with great apes investigated behaviours resembling human disgust and found that nonhuman apes avoid bad-tasting food and pungent odours, they wash or wipe preferred food in case it was previously contaminated, and they try to avoid the body excretions of conspecifics. However, the study did not find sufficient evidence of disgust-like behaviour towards sick conspecifics (Case et al. 2020). Moreover, it has been found that while food contaminated with faeces or dirt tends to be avoided by chimpanzees, some individuals will still eat it (Sarabian et al. 2018). Yet, it should be noted that in humans, culture and the cognitive component of the BIS play an important role and these aspects are rarely studied in nonhuman mammals, although they cannot be excluded as non-existent.

Recent studies on pathogen avoidance are usually formulated within the conceptual framework of the BIS (Kavaliers and Choleris 2018, Sarabian et al. 2017, Poirotte et al. 2019). This theoretical framework was originally formulated in human research – and applied in the context of mate choice – but the underlying mechanisms seem to have a broader applicability to other mammals and contexts. In the following, we thus do not limit ourselves to the context of mate choice: we assume that pathogen avoidance is an intrinsic response to an organism's ecology.

The main aim of this chapter is to review the role of chemical cues in pathogen avoidance in mammals. In the introduction, we focused on the proposed mechanism of such pathogen avoidance, namely the BIS. Now we will turn to the possible causes of changes in body odour during infection and to the perception of chemical cues in both nonhuman and human mammals. Having this in place, we shall review the physiological mechanisms, hormonal influences, and ontogeny of avoidance behaviour.

2 Disease-related changes in the body odour

Many infectious diseases are accompanied by characteristic changes in body odour. There are several possible ways (and their combinations) in which this can happen. First of all, an ongoing infection can change the metabolic circuits as a direct effect of pathogen itself, for example a skin infection. These metabolic changes can be subsequently perceptible in the body excretions, such as urine, faeces, breath, or sweat from different areas of the body (Havlíček et al. 2017, Shirasu and Touhara 2011). Secondly, an ongoing infection can change the composition of the microbiome, which can in turn alter the odour quality

(Schellinck et al. 1991, Penn and Potts 1998). Skin microbiome in particular changes throughout life and might be able to reflect rapid changes in metabolites (Li et al. 2019).

Thirdly, and perhaps most importantly, activation of an immune response can lead to changes in the body odour. Although the details of particular mechanisms remain largely unknown, we assume that immune response activation (leading for instance to an inflammation) affects the metabolic circuits. Volatile products of such metabolic changes can be perceptible in body excretions. Lipopolysaccharide (LPS), widely used in studies on disease-related changes in odour, may serve as an illustrative example of this phenomenon. LPS is a major component of Gram-negative bacteria's surface membrane, and it highly effectively triggers the innate immune response. It is the main component encountered by the immune system and it plays a significant role in pathogenicity, i.e., an organism's ability to cause a disease (Bertani and Ruiz 2019). Injection with an antigen, such as the LPS, can activate a cascade of metabolic immune reactions and these metabolic changes may in turn have an impact on the substances which bodies excrete in urine, faeces, or sweat. In rodents, it has been shown that the production of darcin (a mice male sex pheromone) and other urinary proteins drop significantly after activation of the immune system (e.g., Litvinova et al. 2005, Lopes and König 2016). Nevertheless, the particulars of the mechanisms which produce these immunity-related metabolites in excretions are still mostly unknown.

Finally, an immune response to infection can also lead to changes in the levels of steroid hormones, which can likewise affect body odour. Androgens, mainly testosterone, have an immunosuppressive effect (Folstad and Karter 1992), which is why it might be adaptive to decrease their levels during infection (Wingfield et al. 2001, Trigunaite et al. 2015). The decrease can be adaptive because the energy thus spared can preferentially be used for immune responses, which increases the likelihood of success in fighting the pathogen (Wedekind and Folstad 1994). Still, the details of association between testosterone and immune system remain unclear (for a review, see Roberts et al. 2004). It has been observed that in male mice whose immune response was activated by an injection of sheep red blood cells (SRBC), concentrations of major urinary proteins (MUPs) had significantly dropped (Litvinova et al. 2005), whereby it is relevant to note that MUPs bind to and stabilise a variety of volatile scent substances. In this manner, they are responsible for the release of pheromones in air from urine scent marks (Brennan and Kendrick 2006). The drop in their concentration led to a decrease in scent attractiveness mainly in both castrated and control males treated with SRBC. In contrast, in males treated with high levels of testosterone, no decline in scent attractiveness was observed (Litvinova et al. 2005). Similarly, LPS-treated male mice had significantly lower testosterone levels than controls and were less preferred by females. Moreover, after immunoactivation, male mice were less active and their vocalisation patterns had changed (Lopes and König 2016). LPS injections led to an increase in corticosterone and decrease in testosterone in male rats, but the avoidance response to the odour of LPS-injected males was not associated with changes in the abovementioned hormones (Arakawa et al. 2010a).

Levels of stress hormones can also change during an ongoing infection. For example, the influenza virus in infected mice increases corticosterone levels in the plasma (Dunn et al. 1989). In mice, even viruses with a different host, such as the Newcastle disease virus, led to an increase in corticosterone levels (Smith et

al. 1982). In humans, cortisol levels likewise increase during an infectious disease, as documented for instance by Reichenberg et al. (2001) in their experiments which used bacterial endotoxin.

Nevertheless, the results of studies which investigated the association between steroid hormones and body odour qualities in healthy humans are inconsistent. For instance, one study showed a positive association between cortisol levels and perceived odour attractiveness (Rantala et al. 2006), while another study reported a negative relationship (Butovskaya et al. 2013), and yet other research showed that cortisol does not predict body odour attractiveness in men at all (Thornhill et al. 2013). Similar discrepancies have been reported for the association between the quality of body odour and testosterone levels (Rantala et al. 2006, Thornhill et al. 2013). Clearly, further research is needed to disentangle these inconsistencies and enable some robust conclusions about infection-related changes in body odour mediated by steroid hormones.

Interestingly, not just the levels of steroid hormones but also scent-marking as such can provide (in mice) sufficient cues to current health status. Males infected with *Salmonella enterica* have reduced the area of marking, although the total number of scent marks did not differ from noninfected individuals (Zala et al. 2004). The infection can affect behaviour in a broader sense, by activating so called 'sickness behaviour', which is triggered by pro-inflammatory cytokines produced by activated neutrophils and macrophages after contact with pathogens (Konsman et al. 2002). This set of adaptive behavioural changes consists of decreased food intake, social withdrawal, lethargy, and overall decrease in motor activity (Szentirmai and Krueger 2014).

Odour changes have been considered merely a side effect of pathogen activity and host's response. But it might also be a direct adaptive activity of the pathogen (Poulin 1995). According to the manipulation hypothesis, pathogens purposefully and specifically alter the behaviour or attractiveness of their hosts so as to increase the likelihood of their transmission to an uninfected host. For instance, it might be advantageous to attract an insect vector using an attractive chemical stimulus. In the case of malaria, the parasite (*Plasmodium falciparum*, which is transmitted by the *Anopheles* mosquito) increases attractiveness of the hosts' odour for the vector, apparently to facilitate parasite transmission to other hosts (Lacroix et al. 2005). To the best of our knowledge, molecular mechanisms are unknown, but *P. falciparum* produces a repertoire of plant-like volatile compounds (parasite-produced terpenes), which may be responsible for the higher attraction of vector mosquitoes to the hosts (Kelly et al. 2015). A change in odour can also be a warning that the host is already infected and another parasite should search for another host, because otherwise they would compete for a single source (Prugnolle et al. 2009).

3 Detection of pathogen threat

Detection of a potential source of threat is a crucial component of the BIS, without which a subsequent avoiding reaction cannot be executed. Assessment of pathogen threat from conspecifics or a potential source of contagion in the environment can rely on various visual, acoustic, olfactory, gustatory, and tactile cues, or their combinations.

Although for humans, the visual modality is undeniably important in assessing the current health status of other people, for various nonhuman mammals it is olfaction that plays the key role. The benefits of chemical perception include its ability to detect at relatively long distances, which decreases the likelihood of pathogen transmission. Moreover, the relative stability of sources: some substances, depending on their chemical properties such as volatility and degradability, can remain perceptible even for several days. Olfaction is especially useful in dark and noisy environments, where visual and auditory perceptions are limited, while chemical substances can persist and remain detectable even in sensorily overloaded environments (Wyatt 2003).

3.1 Detection and discrimination of pathogen threat in nonhuman mammals

Various nonhuman animals can detect infected conspecifics and tend to prefer the healthy over the infected ones. So far, most studies on this subject have been conducted on rodents. It should be further noted that a majority of studies on the discrimination between healthy and infected conspecifics were performed in the context of mate choice. Still, avoidance of a sick conspecific is relevant in other social contexts as well, and it extends to same-sex individuals and juveniles. Another consequence of the abovementioned focus on mate choice is that female rodents are disproportionally more often used as assessors because of their high level of mate selectivity.

The detection of and reactions to odour cues of infected rodents are usually tested by a preference test (e.g., total time spent next to the odour sample) or by avoidance behaviour. Preference and investigation tests are conducted mostly in Y-mazes or in special chambers where individual animals are released and their behaviour recorded. Animals can sniff odour samples through a wire mesh or through special inner walls between the prepared chambers, whereby samples take the form of soiled bedding or collected urine and associated secretions presented on a piece of filter paper. The target individuals are experimentally infected with a pathogen, while control individuals are treated with a saline or a water solution.

In a study addressing this issue, female mice preferred as their first choice, spent more time investigating, and made more contact with samples of urine and associated secretions of noninfected male mice (n = 15) compared to samples from males infected with *Eimeria vermiformis* (n = 15) (Kavaliers and Colwell 1995a). *E. vermiformis* is an intracellular protozoan which infects the small intestine epithelium in murine species; their oocysts are shed in faeces and can infect other individuals during feeding and grooming (Figueiredo-Campos et al. 2018). In most animals, the infection is asymptomatic but, depending on the infection dose, can cause diarrhoea (Perrot-Minnot and Cézilly 2010). Samples were collected 5 days after infection, while oocyst shedding starts on day 8, with maximum output around day 10 after infection (Kavaliers and Colwell 1995a). The same effect was observed in another study which compared sham-infected males and males infected with *E. vermiformis*. Preferences of oestrous females (n = 5 per group) for a blank odour sample compared to an infected male sample were dependent on the stage of infection. Pre-infective males on day 4 of infection were preferred to blank odour samples; around the onset of infectibility (on day 7) there was no significant preference for either odour sample and on day 10, when individuals were highly infective, preference for the infected males had significantly dropped (Kavaliers et al. 1997). A

similar pattern was found even when the infection agent was a nonreplicating strain of *Salmonella enterica*, a gram-negative, intracellular bacteria which causes gastroenteritis, fever, and headaches, but can also lead to a serious infection of the central nervous system (Wickham et al. 2007). Females (n = 28) preferred the urine odour of sham-infected males (n = 16) to the urine odour of experimentally infected males (n = 19), although the initial choice or number of visits did not significantly differ (Zala et al. 2004). In another study, female rats spent more time with urine odour samples from sham-infected males (n = 30) compared to males infected with *Hymenolepis diminuta* (n = 39), but the first-visited arm of the maze was not different than expected by chance (Willis and Poulin 2000). *H. diminuta* is a cestode living in the small intestine of rats; it reduces their digestive efficiency, and its intermediate host are coprophilic arthropods. From these results, one can conclude that naïve individuals (i.e., who did not come into earlier direct contact with an infected conspecific), display a relatively straightforward preference for healthy conspecifics.

Preference for healthy individuals can be modulated by various factors, including previous experience and the mating system (Kavaliers et al. 2003a, Klein et al. 1999). For example, preferences can be altered by a brief pre-exposure of females (n = 45; 15 per condition) to the urine odour of male mice infected with *Heligmosomoides polygyrus* (Kavaliers et al. 2003a), a naturally occurring intestinal helminth parasite of rodents with a direct life-cycle and high infectability (Johnston et al. 2015). In mice, it leads to a chronic infection where multiple immunomodulatory mechanisms suppress the host's immune response (Maizels et al. 2012). The initial choice and overall preference were in favour of the urine odour of sham-infected males, but when females were pre-exposed to the odour of an infected male, they subsequently preferred that odour (Kavaliers et al. 2003a). Interestingly, when female mice are pre-exposed to cadaverine (n = 20), a substance that can serve as a cue to the presence of a decaying corpse, they subsequently decrease the number of contacts with males injected with LPS (n = 17), which activates the immune response of an organism (Renault et al. 2008). It seems therefore that environmental odour cues can significantly modulate social contacts and possible mate choice.

Furthermore, the urine odour of male mice infected with murine louse (*Polyplax serrata*) (n = 5) was less preferred than the urine odour of clean males (n = 5) on two consecutive days, regardless of familiarity with the odour of louse-infected males (Kavaliers et al. 2003c). *P. serrata* is a louse of mice and rats and the relatively low level of infestation (Murray 1990), which has been controlled for in the study, did not cause in the mice any symptoms, such as weight loss or poor grooming. The females (n = 5 per condition) preferred the odour of urine collected from new and clean males on the first day and their preference did not change on the second day of the experiment although the louse-infected males' odour was familiar. In short, when presented with two new clean males on the second day, they preferred the odour of the new individuals (Kavaliers et al. 2003c). It seems therefore that familiarity with urine odour may play a role in the preference, but this depends on the type of parasite and its infectability. Moreover, a longer pre-exposure (3 days prior to testing) to the urine odour of an unfamiliar or infected male with *H. polygyrus* enhanced in the females (n = 10 per condition) their subsequent discrimination against the odour of infected males. Female mice were pre-exposed for 15 minutes to the odour of urine collected from an infected familiar male, infected unfamiliar male, uninfected familiar male, and uninfected unfamiliar male, or to the odour of clean

bedding. Females displayed a significant preference for the urine odour of uninfected males regardless of familiarity with the pre-exposed male, whereby the odours of an unfamiliar or infected male enhanced the abovementioned discrimination against the odour of infected males (Kavaliers et al. 2014). In mice, familiarity via pre-exposure to an odour seems to be an important modulating factor of preference. When females are briefly pre-exposed to a male urine odour, they later tend to prefer the familiar odour regardless of the infection status of the male. On the other hand, a longer pre-exposure can enhance the discrimination against the odour of infected males.

Interestingly, the females of prairie voles (*Microtus ochrogaster*) (n = 20) and meadow voles (*Microtus pennsylvanicus*) (n = 20) differ in their odour preference when choosing between the urine odour of males either not infected (n = 20 per species) or infected with *Trichinella spiralis* (n = 20 per species) (Klein et al. 1999). *T. spiralis* is a nematode parasite which causes trichinosis in a wide range of vertebrate species, whereby the infection is often asymptomatic (Golab et al. 2009). Female meadow voles spent more time with the bedding from noninfected males, while female prairie voles spent a comparable amount of time with the bedding of both infected and noninfected males (Klein et al. 1999). It should be noted that meadow voles are polygynous, while prairie voles are a monogamous species. This suggests that species with polygynous and promiscuous mating systems (such as mice or rats), which are more susceptible to pathogen transmission, are more sensitive to various markers of the health status of potential partners. In another experiment, male mice (n = 20) were less likely to mount and copulate with female mice infected with *T. spiralis* (n = 20) than with uninfected females (n = 10). On the other hand, when males experienced and unexperienced in terms of previous copulation were observed separately, the experienced males showed significantly more investigatory activity towards infected females, while responses of the unexperienced males were not affected by infection in the females (Edwards and Barnard 1987). The preference for uninfected individuals is evident, but factors such as the previous experience are important as well. Experienced individuals seem to be more cautious about approaching an infected conspecific, but it is not known whether this caution is reflected in reproductive success.

It has been shown that the influenza virus decreases the attractiveness of male urine odour to female mice: females (n = 80) spent more time investigating post-infection and pre-infection odours than odours collected from currently infected males (n = 15) (Penn et al. 1998). On the other hand, male mice (n = 30) infected with a subclinical dose of tick-borne encephalitis virus (viral RNA was detected after RT-PCR control) had higher levels of blood testosterone, and oestrus females (n = 90) spent more time with their bedding than with the bedding of males from a control group (n = 30) who received a saline vehicle (Moshkin et al. 2002).

In rodents, odour can be affected not only by parasites or viruses. Experiments that used a non-replicating bacterial strain (Zala et al. 2004) or foreign antigens (sheep red blood cells) (Moshkin et al. 2002) provide evidence that immune activation on its own can reduce the attractiveness of male urine odour. Similarly, activation of the immune system by an LPS treatment also alters the quality of odour. Odour samples from male rats treated with LPS (n = 24) were less sniffed and their bedding was more frequently buried and avoided by both female (n = 9) and male rats (n = 18) (Arakawa et al. 2009). In another study,

male rats (n = 10) also exhibited an aversive response to LPS-treated males (n = 10); this was expressed by reduced sniffing, increased overall time spent on the other side of the cage, and by burying the bedding (Arakawa et al. 2010). This pattern was not, however, observed in prepubertal males in whom the level of sniffing or avoidance did not differ between LPS-treated conspecifics (n = 4) and the control group (n = 4) (Arakawa et al. 2009). Furthermore, the aversive properties of urine odour collected from LPS-treated individuals (n = 12) were blocked by treatment with interleukin-10 (IL-10), an anti-inflammatory cytokine central to the inflammatory processes involved in the production of illness-related urinary odour cues (Arakawa et al. 2010a). A similar effect has been demonstrated in mice treated with keyhole limpet hemocyanin (KLH), a T-cell-dependent antigen commonly used in mammals and birds. KLH-treated males (n = 19) were less preferred, which manifested itself in fewer sniffs and shorter sniff duration. Interestingly, males with a stronger immune response (antibody response) were preferred to males with a weaker reaction to KLH (Gerlinskaya et al. 2012).

As shown above, most studies on discrimination between infected and healthy individuals were conducted in laboratory conditions. Data based on wild vertebrate populations are rare but show similar patterns of preference in favour of less parasitised conspecifics. Temporarily isolated mandrills (n = 25) spent less time in the proximity (less than one meter) of a highly parasitised faecal sample than in the proximity of a less parasitised faecal sample (Poirotte et al. 2017). It seems therefore that mandrills can discriminate, based on faecal odours, between more and less parasitised social partners. Wild mandrills also avoided grooming conspecifics who had been infected with orofecally transmitted parasites, mainly *Balantidium coli*, which causes dysentery. Another evidence shows that the feeding behaviour of chimpanzees (*Pan troglodytes troglodytes*) (n = 20) is influenced by possible contaminants, mainly conspecific faeces. Animals were less likely to feed, and tended to leave the area, when food was associated with the odour of faeces. On the other hand, their feeding behaviour did not differ when they were exposed to the odours of blood or semen (Sarabian et al. 2017), which need not be linked to pathogen avoidance but rather to antipredator behaviour or reaction to conspecific aggression. This possibility was explored in a study where female Holtzman rats (n = 15) escaped faster from the experimental area where they were exposed to the blood of rats or mice compared to a slower reaction to human blood or distilled water (Hornbuckle and Beall 1974).

But not all studies show avoidance of infected individuals. In the highly social species of banded mongooses (*Mungos mungo*) (n = 6 troops, 5 to 65 individuals per troop), a study found no avoidance of individuals with clinical signs of disease (caused by *Mycobacterium mungi*) during focal observations, although the sick banded mongooses showed typical sickness behaviour, such as reduced activity, which may serve as a sufficient cue (Fairbanks et al. 2015). Moreover, mongooses have a very good sense of smell, which helps them identify individual conspecifics. Nevertheless, it should be noted that *M. mungi* is a novel pathogen and it is characterised by a unique transmission and exposure routes. It is usually transmitted via infected urine and anal gland secretions, which are used in olfactory communication of banded mongooses (Alexander et al. 2018).

It has been suggested that odour preferences are a reliable proxy to mate choice (Egid and Brown 1989), but other evidence indicates that mate preference and actual reproductive success may differ. In mice, although the initial choice of females (n = 24) favoured sham-infected males, the numbers of offspring did not significantly differ between the sham-infected males (n = 9) and males infected with *S. enterica* (n = 9) (Zala et al. 2015). A similar finding showed that female mice (n = 5 per arena) did not differentiate in their mate choice between sham-infected males (n = 2 per arena) and males infected with *H. polygyrus* (n = 1 per arena) in four arena settings (Ehman and Scott 2004).

From these studies, we can conclude that nonhuman mammals, mainly rodents, are capable of distinguishing between healthy and infected conspecifics in laboratory conditions. These effects are influenced by familiarity of the conspecifics and by novelty of the pathogen in the studied species. It also seems that actual partner choice does not always follow prior odour preferences, because the number of offspring did not differ between healthy and parasitised males. Further studies should test differences between reproductive success, avoidant reaction to the odour of infected individuals, and the effect of pathogenicity. The decision to copulate with a parasitised individual may be flexible and depend on the availability of alternative partners as well as hazardousness and infectability of a particular pathogen. For example, if the risk of contracting the infection is lower than the benefits of possible reproduction, it may be advantageous to copulate with such an individual regardless of its health status.

3.2 Detection and discrimination of sick conspecifics in humans

Experimental studies which investigated human ability to discriminate between body odour samples from healthy and sick individuals show similar patterns as the previously presented studies conducted on nonhuman animals. Moreover, in humans we can investigate affective states, such as disgust. Similar studies in nonhuman animals are rare, although a display of stereotypical facial expressions associated in mice with toxin disgust has been recently documented (Dolensek et al. 2020).

Various diseases are accompanied by changes of body odour: for example, bacterial vaginosis is frequently associated with a fishy odour caused by the production of trimethylamine (Wolrath et al. 2005), while the diarrhoea caused by *Vibrio cholerae* has a specific sweet odour (Garner et al. 2009). Overall, it has been demonstrated that various infectious diseases do affect the odour emitted from skin, breath, urine, or faeces (see reviews by Shirasu and Touhara 2011, Havlíček et al. 2017).

Behavioural tests assessing the attraction to/avoidance of various odours, commonly used in rodent research, are rarely used in human studies. In humans, researchers usually rely on hedonic ratings of odour samples and on self-reporting of affective states. Research has shown that axillary body odours collected from individuals infected with gonococcus (*Neisseria gonorrhoeae*) (n = 13) were rated by female raters (n = 14) as less pleasant and more putrid than samples collected from healthy (n = 16) or recovered donors (n = 5) (Moshkin et al. 2012). Interestingly, samples of recovered donors were more likely to be described as floral. Another study collected samples from donors (n = 23) who were currently sick with a naturally occurring acute respiratory infection (suffering from cough, sore throat, shortness of breath, coryza, fever, headache, malaise, or myalgia). The odours were rated by both sexes (n = 46) as nominally (but not formally

significantly) less pleasant, less healthy, and more intense and disgusting than samples collected from the same donors when they were in full health (Sarolidou et al. 2020b). People are also capable of detecting the early olfactory cues of sickness. Within a few hours after activation of immune system by an LPS injection, body odour samples collected from donors (n = 8) were perceived by raters (n = 40) as less pleasant, less healthy, and more intense than samples from the same donors after a placebo injection (Olsson et al. 2014). Moreover, after LPS administration the natural decrease of averseness of healthy urine was disrupted, and both pyrrole and acetophenone (distinctive urine volatiles used as biomarkers for various diseases) were more abundant in urine collected from LPS-treated individuals (n = 20) than in the urine collected from placebo-treated controls (n = 13). Non-disrupted natural rhythm, meanwhile, is characterised by a decrease in urine odour aversiveness through the day (Gordon et al. 2018).

In a recent study, we activated the immune system by vaccination against hepatitis A/B and *meningococcus* (combination of immunisation against a viral and bacterial disease) in men (n = 21). Interestingly, we found that body odour samples collected 14 days after vaccination were rated by females (n = 88) as more attractive, healthier, and less intense than samples collected before vaccination (Schwambergová et al. submitted). In contrast to previous studies based on the use of LPS, we did not perform the rating shortly after vaccination but two weeks after it. It is therefore possible that a drop in odour attractiveness is followed by its increase – and our study captured only the latter. The mechanisms of these alterations remain unknown and future studies should investigate their dynamics in more detail.

Besides the direct effect of odour collected from sick individuals on the perceiver, chemical cues may also have modulatory effects. When participants were presented with a facial photograph simultaneously with body odour collected after LPS treatment, their ratings of liking the presented person had significantly decreased (Regenbogen et al. 2017).

Although there are only a handful of studies on the discrimination of health status based on odour cues in humans, we can tentatively conclude that they show similar patterns as in nonhumans, namely preference for healthy individuals. This suggests that pathogen disgust could be the central motivational component of avoidance behaviour, which is what is proposed by the theoretical framework of the BIS (reviewed in Oaten et al. 2009, Kavaliers et al. 2020). Responses of the BIS include not only the affective experience of disgust but also the activation of aversive cognitions into working memory. Subsequently, the motivational system guides the behavioural response to minimise the risk of infection (e.g., leaving a crowded room with sneezing individuals) (Schaller and Park, 2011). Future studies should employ experimentally induced avoidance behaviour, for example in a room with unpleasant smell, and measure disgust to uncover the possible mechanisms of the behavioural response.

3.3 Multisensory integration

Most studies on pathogen avoidance focus on a single modality, but in everyday life individuals are often exposed to stimuli that can be perceived by multiple senses. An interplay of cues from various modalities significantly increases the likelihood of detection and adequate evaluation of potential danger. Moreover, a response can be amplified (*multisensory enhancement*), meaning that a response to stimuli from multiple

modalities exceeds the response to either of the stimuli presented alone (Stein and Stanford 2008). This is attested, for instance, by responses to jointly presented odour of faeces and a depiction of faeces, or a decrease in reported liking of a person when the facial photograph and 'sick' body odour are presented together (Regenbogen et al., 2017). Moreover, the multisensory percept can be qualitatively different from the mere sum of the individual components and convey unique information. On the other hand, a decrease in response (*multisensory depression*) implies that response to cross-modal stimuli is weaker than the response to the most effective of its component stimuli (Stein and Stanford 2008). For example, because visual cues create expectations of flavour, when the colour of salsa is experimentally adjusted to be less red, thus in our eyes less spicy, the spiciness rating after tasting the product is also depressed (Shermer and Levitan 2014).

To the best of our knowledge, studies conducted on nonhuman animals have not yet investigated the multimodal perception of infection cues. What we do know, however, is that it is not only the urine odour of male mice that changes its quality after LPS treatment. Behavioural changes in injected individuals also play a significant role. For example, males (n = 21) were also less active, less motivated to engage with females, and they changed their vocalisation patterns after LPS injection (Lopes and König 2016). This indicates that there was a variety of cues available, although the olfactory one might be most important. A previous human study asked participants (n = 30) to indicate their liking of a person whose picture was displayed while their body odour was simultaneously presented during an fMRI session (Regenbogen et al. 2017). Facial photographs after LPS treatment were rated as less likeable, less healthy, less attractive, and less desirable for social interaction, and the ratings were even lower if that person's odour after LPS treatment was presented at the same time. Sickness status also increased the neural activation of odour perception networks, specifically the superior temporal sulcus, which is a core area for multisensory integration, and the orbitofrontal cortex, which is part of the olfactory network. In another study, participants (n = 77) were exposed to facial pictures and body odours from the same individuals (n = 22) either before or after the LPS treatment. Both facial images and odour collected after LPS treatment were rated as less likable, but facial electromyography showed no activity of muscles that would indicate a disgust response (Sarolidou et al. 2020a). Future studies should, however, put more emphasis on the multisensory perception of cues related to the health status to test their interactions and relative importance.

4 Physiological mechanisms of avoidance behaviour

In the previous sections, we have reviewed preferences for and discrimination between odours from infected and healthy individuals. In the following, we focus on the physiological and/or affective reactions of odour perceivers. In animal research, studies of affective states are mostly limited to stress or fear reactions. Humans, on the other hand, can report their feelings (e.g., how attractive or disgusting they find a particular odour) and thus provide data that cannot be collected from nonhuman animals, where researchers must rely on indirect indicators. The most common method for measuring physiological reactions in rodents is the level of analgesia (reduced pain sensitivity). Pain sensitivity is usually assessed on a hot plate (50-60°C), where vigorous foot flutter or licking is recorded. Subsequently, researchers observe thermal latencies. Analgesia is a defensive response to threatening stimuli: it helps the organism to prepare for a fight or flight

reaction. It has been found that exposure to the odour of urine of parasitised males induced analgesia in females and their reaction differed depending on the length of exposure (Kavaliers et al. 2000). Prolonged exposure (around 15 minutes) induced an endogenous opioid-mediated analgesia, which is associated with stress responses, while a short exposure (under 1 minute) elicited a shorter non-opioid-mediated analgesia associated with anxiety-related anticipatory defence reactions.

Previous studies showed that female mice (n = 6 per case) exposed to the urine odour of males infected with *E. vermiformis* (n = 15) displayed higher thermal response latencies, which were measured as delayed vigorous foot flutter or lick of foot when put on an experimental hot plate. The latency lasted at least 15 minutes after a 30-minute exposure to the odour of parasitised males. Maximum analgesic responses were induced by the odour of pre-infective (5 days after infection) and infective males (10 days after infection) (Kavaliers and Colwell 1993). The results were similar when female mice were exposed to the urine odour of males infected with *H. polygyrus* (n = 30) compared to sham-infected males. Once again, females (n = 5 per case) showed higher latencies and a longer exposure to odour of infected males led to a greater analgesia (Kavaliers and Colwell 1995b). On the other hand, when female mice (n = 5 per case) were infected with the same parasite as the males, their analgesic response to the odour of urine of infected males was reduced compared to exposure to physically stressed males, which normally leads to a lower analgesia than the urine odour of infected males (Kavaliers et al. 1998a). The lower analgesic response to physically stressed males suggests that an infected conspecific may represent a higher threat. Analgesic responses are also influenced by the level of sexual experience. Specifically, sexually experienced male mice (n = 5 per case) displayed a significantly greater level of analgesia compared to sexually naïve males (n = 5 per case) when exposed to the urine odour of females infected with *H. polygyrus* (Kavaliers et al. 1998b). Similarly, prior familiarity lowers analgesia in female mice (n = 15) in reaction to familiar louse-infected males but not to novel louse-infected males (Kavaliers et al. 2003c). These results indicate that rodents find the odour of parasitised conspecifics threatening and/or stressful, whereby lower pain sensitivity may facilitate a subsequent escape. Mice are also capable of maintaining their behavioural avoidance based on prior experience (familiarity) with infected individuals. This may reduce the cost associated with prolonged stress, which is immunosuppressive, as opposed to acute stress, which may temporarily boost immune response (Elenkov and Chrousos 1999). Future studies should test physiological response during experiments in arena settings, where mice may be in direct contact. This approach may help to disentangle the importance of trade-offs between approaching (risking the contagion) and fleeing from a potentially harmful situation. Moreover, it would be beneficial to collect blood samples before and during the experiment to monitor the levels of cytokines and hormones.

4.1 Hormonal influences on pathogen avoidance

The processing of social information responsible for subsequent pathogen avoidance depends on various neurobiological and endocrinological regulatory mechanisms (Choleris et al. 2009). Social recognition and discrimination are crucial for distinguishing individuals who represent a potential pathogen threat. For example, unfamiliar individuals may carry novel pathogens and thus represent a higher health risk. The most

prominent hormones which play a role in social recognition and pathogen avoidance are oxytocin, oestrogens, arginine-vasopressin, and testosterone. Interplay between these hormones allows individuals to respond rapidly and appropriately to social information derived from potential pathogen threats (Kavaliers and Choleris, 2018).

Oxytocin is a nonapeptide hormone facilitating prosocial and sexual behaviours in mammals (Veening et al. 2015). Oxytocin and its receptors play a role in modifying cognitive and motivational processes in reaction to social environment. In this way, oxytocin mediates approach and avoidance of social information (Shamay-Tsoory and Abu-Akel 2016). Mice in whom the gene responsible for oxytocin production was knocked-out failed to recognise familiar conspecifics after a repeated exposure, and that despite normal olfactory and learning abilities (Ferguson et al. 2000, 2001). It has also been demonstrated that mice which received antisense DNA targeted against oxytocin receptors were as impaired in social recognition as were mice with knocked-out gene for oxytocin (Choleris et al. 2003).

The importance of oxytocin in pathogen avoidance has been demonstrated in studies on mice with a knocked-out oxytocin gene. Female mice with this condition did not show preference for the urine odour of either healthy or louse-infected males. In contrast, wild type females and females heterozygous in the oxytocin gene (n = 20 per genotype) exhibited a clear initial preference for healthy males. Females whose oxytocin gene has been knocked-out were also unable to distinguish between novel and familiar males regardless of their health status, and they displayed lower analgesic responses (Kavaliers et al. 2003b). It should be noted that females with a knocked-out oxytocin gene showed normal associative olfactory conditioning to predator odours and were capable of distinguishing between the urine odours of intact and castrated males (Kavaliers et al. 2003b) or stressed vs. non-stressed males (Kavaliers et al. 2003b). These results suggest that oxytocin plays a specific role in pathogen avoidance, but not in general olfactory associative learning.

A similar pattern was found when females (n = 20 per genotype) were choosing between the urine odour of healthy males and males infected with *H. polygyrus* (Kavaliers et al. 2003b). It can thus be concluded that at least one copy of the gene which encodes oxytocin is sufficient for discrimination between healthy and infected individuals and for a modulation of subsequent analgesic responses and avoidance behaviour. Deletion of the oxytocin gene leads only to slight analgesic responses. This was demonstrated by the fact that female mice with knocked-out oxytocin gene were unable to modulate their analgesic response: it remained on the same level and these mice did not perform increased investigation of urine odours of novel mice. On the other hand, wild type female and heterozygous female mice (n = 20 per genotype) are able to modulate their analgesic responses based on prior familiarity: they display a reduced analgesic response to the odours of familiar males and an enhanced response to novel males (for review, see Kavaliers and Choleris 2018).

In humans, researchers often use oxytocin nasal spray, which has an impact on social cognition and early detection of emotionally charged social cues (see Graustella and MacLeod 2012). Oxytocin also enhances the aversive response to threat presented in the form of negative facial expressions, whereby the response is stronger in women (Luo et al. 2017). To the best of our knowledge, there is as yet no study that

tested the effect of oxytocin on the ability to discriminate between odours based on the health status in humans.

The processing of social information is also dependent on oestrogens, partly due to their modulating effect on oxytocin. It has been shown that knocking out genes for ER-α and ER-β receptors impairs social cognition and behaviour. Social recognition in ER-α knocked-out mice was impaired to a comparable degree to mice with knocked-out genes for oxytocin. Oestrogens acting through the ER-β can directly regulate oxytocin gene expression, but social impairment in this condition is only partial (for a review, see Ervin et al. 2015). Choleris et al. (2003) proposed that oestrogens regulate oxytocin secretion in the paraventricular nucleus of the hypothalamus through the ER-β. Through the neurons of the paraventricular nucleus, oxytocin reaches the amygdala where oestrogens regulate the expression of oxytocin receptors through the ER-α. In fact, it has been shown that male mice knocked out in ER-β and ER-α displayed impaired recognition and aversive responses to the urine odour of males infected with *H. polygyrus* (Kavaliers et al. 2004).

Another important component of olfaction-mediated social recognition is nonapeptide arginine-vasopressin. Male rats (n = 8 per group; 3 groups) exposed to the urine odour of a conspecific with immune system stimulated by LPS administration (Arakawa et al. 2010b) activate their arginine-vasopressin receptors, mainly in the medial amygdala. Arginine-vasopressin appears to be more essential for social recognition in males than in females, because its expression is usually higher in male brains. Oxytocin and arginine-vasopressin can interact with the same three types of receptors (OT, V1A, and V1B) (Choleris et al. 2009). It should be noted, though, that the main effect of abovementioned hormones is not in its separate contribution to social recognition but rather in the interplay between arginine-vasopressin, oxytocin, and the sex hormones (such as oestrogens or testosterone). Oestrogens facilitate social recognition through their receptors by regulating oxytocin, while arginine-vasopressin is regulated by testosterone, which is why the effects of androgens on social recognition could be mediated by arginine-vasopressin, but the exact mechanism is yet to be discovered (see Gabor et al. 2012).

The compensatory prophylaxis hypothesis (CPH) suggests that progesterone enhances pathogen avoidance, in particular disgust, in women (Fessler et al. 2005). Disgust sensitivity is expected to be high when progesterone levels are elevated, for instance during pregnancy when the physiological immune system is downregulated to prevent it from rejecting the embryo. Moreover, during the mid-luteal phase of the menstrual cycle, when progesterone levels are high, disgust sensitivity is higher than during the menstruation phase (n = 30) (Żelaźniewicz et al. 2016). The same pattern was observed for pathogen disgust in women in their luteal phase who lately had an infection (n = 75) but not in healthy women (n = 477) (Milkowska et al. 2019). In early pregnancy, the decreased levels of pro-inflammatory cytokines are compensated by an increase in contamination disgust (Kaňková et al. 2022). Female mice treated either with allopregnanolone, a metabolite of progesterone, or with a low or high dose of progesterone (n = 10 per group), spent significantly less time near the odour of males (n = 30) infected with *H. polygyrus* (Kavaliers et al. 2021, Bressan and Kramer 2022).

We can conclude that avoidance behaviour is affected by a coordinated action of oxytocin, vasopressin, oestrogen, and testosterone. To fully capture the proximate mechanisms of pathogen avoidance,

future studies should also consider progesterone, and test its interactions with the abovementioned hormones. The interaction between arginine-vasopressin and androgens likewise deserves further investigation, especially in association with avoidance of potential threat.

4.2 Association between the BIS and the PIS mechanisms

The BIS and the PIS do not operate independently: they tend to modulate each other. After exposure to a possible source of contamination, the activation of the BIS may lead to a preparation of the PIS (Schaller et al. 2010). Interactions between these two systems involve opioid compounds, which are responsible for greater analgesia after a longer exposure to odours of infected males but have also immunomodulatory properties. They may thus play a role in an adaptive preparation of the PIS in case of subsequent pathogen transmission (Stefano et al. 1996). Another study has shown that after injecting rats with LPS their social partners (n = 32) displayed modulated cytokines responses. In particular, they displayed increased levels of Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α) (Hamasato et al. 2017). Cytokine IL-1β is a key mediator of the inflammatory response produced by cells of the innate immune system and a similar role is played by IL-6, which is likewise pro-inflammatory and thus active during inflammation, while contributing to the maturation of B cells. TNF is a pro-inflammatory cytokine with an important role in cell survival, proliferation, differentiation, and death (Gulati et al. 2016). All these cytokines display activity during the preparation phase of the PIS in organisms that come into proximity of a potential source of contamination, such as a sick conspecific or body secretions thereof.

Disgust, a widely studied aspect of human psychology, has an effect analogous to opioid analgesia in rodents. Persons (n = 32) who were exposed to disgusting pictures depicting food or non-food stimuli had a higher core body temperature and manifested an oral inflammatory response, increased levels of proinflammatory cytokines (salivary TNF-a and albumin), and a downregulation of immunoglobulin A (IgA) (Stevenson et al. 2012). This suggests that a potential pathogen threat may lead to a specific immune response responsible for sufficient preparation of the immune system. On the other hand, the upregulated production of salivary IgA may be lost as saliva is flushed from the gaping mouth. It has been hypothesised that disgust evolved from distaste in terrestrial vertebrates and that it is responsible for spitting out potentially harmful and toxic substances (Rozin et al. 2008). Moreover, when participants (n = 28) were exposed to photographs depicting symptoms of an infectious disease, they produced significantly more proinflammatory IL-6 than a group exposed to photographs depicting guns as a control threat (Schaller et al. 2010). Recently, Juran et al. (2023) showed that disgusting odours as compared to neutral odours elevate TNF- α levels.

These results indicate that the BIS may also prepare the organism for subsequent infection after exposure to potential contaminants. The abovementioned opioid peptides have immunomodulatory properties and contribute to communication with the PIS; they thus play a role similar to IL-1β, IL-6, and TNF-α, which show elevation after contact of healthy rats with LPS-treated conspecifics or after exposure to images depicting potential harmful and disgusting stimuli in humans. Future studies should address this issue and test whether olfactory cues to diseases have an effect similar to that of visual stimuli depicting symptoms

of infectious disease. Moreover, one could monitor the strength of reaction when participants are exposed to multimodal stimuli compared to solely visual or olfactory stimuli.

5 The ontogeny of pathogen avoidance

To the best of our knowledge, there is currently no direct evidence regarding the development of olfaction-related pathogen avoidance. It seems that pathogen avoidance depends on learning via interaction with conspecifics and the environment. In naïve mice, for instance, exposure to biting flies led to heightened analgesia and self-burying to avoid being bitten. One day later, the biting parts of flies were removed but mice behaved just as they did the day before, although they could not be actually bitten. Moreover, naïve mice which witnessed other mice being attacked by biting flies exhibited analgesia and self-burying as well (Kavaliers et al. 2001). Early learning might work on the same principle. Still, the learning of pathogen avoidance might take place rather early and could be to some extent independent of any previous experience. For instance, it has been shown that even prepubertal, 21 day-old, rats naïve to the 'infection' odour were able to distinguish between LPS-treated and healthy adult rats. They showed a preference in favour of healthy individuals: the LPS-treated ones were sniffed for a shorter time (Arakawa et al. 2009). This initial preference for healthy individuals might be further modulated by familiarity or sexual experience as discussed above. Furthermore, a study on great apes and their willingness to eat contaminated food had shown that juvenile bonobos were less contamination-risk averse than the adults (Sparks et al. 2018).

Young children tend to tolerate the smell of decomposition or faeces, which can be a potential source of pathogen contagion. In the first two years of life, it is common for children to put in their mouths objects most adults consider disgusting, quite regardless of the nature of the object and the possibility of infection. Nevertheless, the caretakers regulate children's activities by guiding them in what they can put in their mouth and what they should avoid (Rozin et al. 1985). From two years on, the perception of pathogen disgust increases: in one study, for instance, images of maggot-like animals elicited disgust and avoidance in 2.5 year-old children (Stevenson et al. 2010). On the other hand, even very young infants show an aversive reaction to certain tastes and smells generally found disgusting by adults, such as butyric acid (Soussignan et al. 1997). These results support the idea that disgust evolved from distaste and that it helps in the identification of potentially harmful and toxic substances that can be subsequently spat out (Rozin et al. 2008).

The ontogeny of disgust and aversive behaviour deserves further intensive investigation. It would be important to obtain longitudinal data to capture the development of the abovementioned responses. Future studies should test the differences in the olfactory avoidance behaviour of adults and prepubertal individuals. Human research should similarly focus on the reactions of infants and pre-school children to odours which are considered disgusting by adults.

6 Conclusions

There is convincing evidence that various mammals are able to discriminate between healthy and infected conspecifics. On the other hand, the reproductive success of infected and healthy males is – at least in

rodents – comparable, which suggests that preferences are also modulated by other factors, such as familiarity or dominance. Instead of simple avoidance, the BIS may rather prepare the individual for potential infection. The odour of an infected conspecific leads to heightened analgesia and increased levels of opioids and immune markers in the perceiver. Future studies should address the mechanisms of odour changes in the emitters, which are still not well understood. Chemical and microbial analyses would be particularly informative. Research should also investigate the interplay between hormones which affect social recognition and pathogen avoidance, as well as functional links between the PIS and the BIS. Similarly, multimodal perception and integration also deserve further investigation. Furthermore, without a better insight into the ontogeny of pathogen avoidance, our understanding of the underlying processes remains greatly limited. Finally, studies should include a variety of mammals to enable wider generalisations regarding pathogen avoidance. Preferentially, such studies should be conducted on wild populations because, as we have seen, odour preference in the laboratory may differ from actual mate choice. The ecology of the animals concerned, in particular their social structure, might also play an important role in pathogen avoidance. In the past two decades, we have clearly witnessed an enormous progress in our understanding of olfaction-mediated pathogen avoidance, but the list of unknowns remains long and we impatiently await further discoveries in this fascinating field.

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Statement of contribution

This is to confirm that Mgr. Dagmar Schwambergová significantly contributed to the following publication:

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She contributed to the conceptualisation, manuscript writing and subsequent revisions.

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

_____

doc. Mgr. Jan Havlíček, Ph.D.
supervisor

Chapter 11

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

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

Žaneta Pátková[1]✉, Dagmar Schwambergová[1], Jitka Třebická Fialová[1], Vít Třebický[2], David Stella[1,3], Karel Kleisner[1] & Jan Havlíček[1]

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

[1]Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic. [2]Faculty of Physical Education and Sport, Charles University, José Martího 269, 162 52 Prague, Czech Republic. [3]Global Change Research Institute of the Czech Academy of Sciences, Bělidla 986/4a, 603 00 Brno, Czech Republic. ✉email: slamovz@natur.cuni.cz

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.

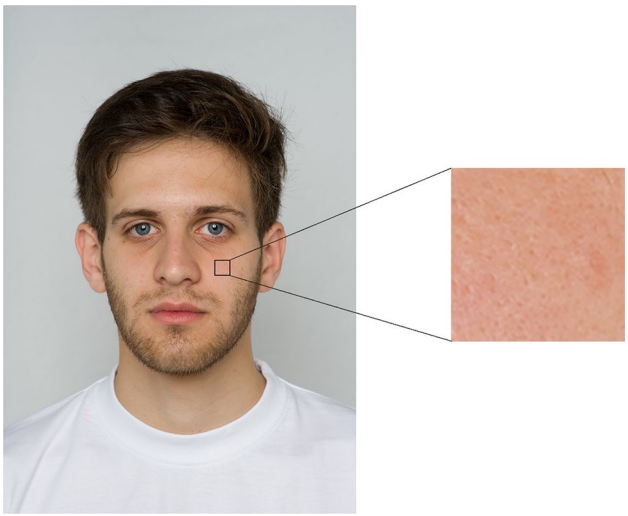

Figure 1. An example of acquired facial photograph with an outlined skin patch on the left and the resulting skin patch on the right (informed consent was obtained to publish the image in an online open-access publication).

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 ($\rho_{\text{Anti-HAV}} = -0.130$, $p = 0.573$; $\rho_{\text{Anti-Mnk}} = -0.078$, $p = 0.738$). 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/l)	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.

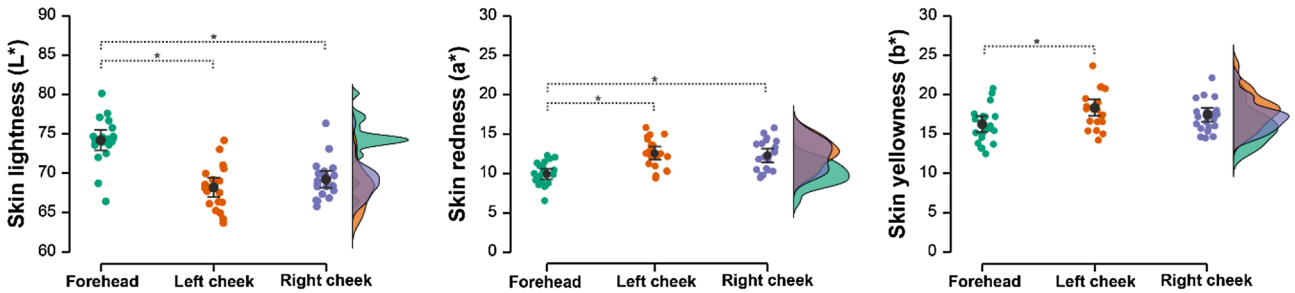

Figure 2. Differences in skin colour (CIE L*a*b*) measured from right and left cheek and the forehead. Black dots represent mean values, error bars show their 95% confidence intervals. Coloured points represent individual data points, while density plots show their distribution. Statistically significant 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., $\geq 80\%$ 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.

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Correspondence and requests for materials should be addressed to Ž.P.

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She contributed to the conceptualisation, investigation, methodology and subsequent manuscript editing and revisions.

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

_____

doc. Mgr. Jan Havlíček, Ph.D.
supervisor

Chapter 12

NO EVIDENCE FOR ASSOCIATION BETWEEN HUMAN BODY ODOR QUALITY AND IMMUNE SYSTEM FUNCTIONING

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No evidence for association between human body odor quality and immune system functioning

Dagmar Schwambergová [a,b,*], Agnieszka Sorokowska [c], Žaneta Slámová [b,d], Jitka Třebická Fialová [a,b], Agnieszka Sabiniewicz [c,e], Judyta Nowak-Kornicka [f], Barbara Borkowska [f], Bogusław Pawłowski [f], Jan Havlíček [a,b]

[a] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic
[b] National Institute of Mental Health, Topolová 748, 250 67 Klecany, Czech Republic
[c] Institute of Psychology, University of Wroclaw, Plac Uniwersytecki 1, 50-137 Wroclaw, Poland
[d] Department of Philosophy and History of Sciences, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic
[e] Smell and Taste Clinic, TU Dresden, Fetscherstrasse 74, 01307 Dresden, Germany
[f] Department of Human Biology, University of Wroclaw, Przybyszewskiego 63, 51-148 Wroclaw, Poland

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ABSTRACT

Previous studies have shown that women perceive male faces with a more reactive immune system as more attractive, but whether body odor might likewise provide cues to immune function has not been investigated yet. These two studies tested a possible relationship between body odor quality and immunoreactivity (Study 1) and immune system function (Study 2).

In Study 1, we collected body odor samples from 21 men just before and two weeks after vaccination against hepatitis A/B and meningococcus. We determined the levels of specific antibodies (selected as markers of immune system's reactivity), testosterone, and cortisol. Subsequently, 88 female raters assessed the odor samples for their attractiveness, intensity, and healthiness. In Study 2, we collected body odor and blood samples from 35 men and women. We assessed key parameters of their innate and adaptive immunity, such as complement activity or total lymphocyte T and B counts and asked 95 raters to assess the odor samples for their attractiveness, intensity, and healthiness.

In Study 1, we found no significant association between antibody levels induced by vaccination and perceived body odor attractiveness, intensity, and healthiness. We also found no significant relationship between antibody levels and steroid hormones (testosterone and cortisol). In Study 2, we likewise found no association between basal key parameters (innate and adaptive) of the immune system and body odor quality. Our results indicate that body odor may not serve as a cue to the reactivity of the immune system.

1. Introduction

Appropriate immune system functioning vitally affects the viability of organisms (for an overview, see (Parkin and Cohen, 2001). Evolutionary theorists therefore proposed that individuals might be attentive to markers of an effective immune system in their mate choice. Selecting a partner with effective immunity would increase offspring's pathogen resistance (indirect benefits) and positively affect partner's health (direct benefits) (Hamilton and Zuk, 1982). A healthier partner might provide better parental care and decrease the chances of transmitting infections to a partner (Kirkpatrick and Ryan, 1991). Still, the immune system is highly complex and various earlier studies operationalized its responsiveness in different ways.

One aspect of immune system functionality that has been assessed in the context of mate choice is immunoreactivity, i.e., the magnitude of organism's response to antigens. Clinical studies show that higher effectiveness of the immune system might be indicated by increased levels of antibodies after vaccination (Burns and Gallagher, 2010). Moreover, antibody levels are considered a reliable marker of underlying immune processes. Previous studies in humans showed that faces of European males with higher levels of antibodies after vaccination against hepatitis B were rated as more attractive (Rantala et al., 2012)

* Corresponding author at: Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic.
E-mail address: schwambd@natur.cuni.cz (D. Schwambergová).

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but an analogous pattern was not observed for female faces (Rantala et al., 2013). In another study, authors experimentally activated innate immune response by injecting a bacterial lipopolysaccharide (LPS) to a sample of South African males whose facial images were then rated for attractiveness. The immune response was determined by C-reactive protein (CRP), a widely used peptide marker for inflammation, and by cytokine levels (interleukins 10, 6, 2, 8, 4; GM-CSF, IFN-γ, and TNF-α), which are important signal peptides that play a key role in immune response to infections and inflammation. The results showed a positive association between perceived facial attractiveness, health, and elevated levels of cytokines. Moreover, a stronger immune response was also linked to yellower skin color, commonly viewed as a cue to higher carotenoid levels (Phalane et al., 2017). Similarly, certain masculine voice characteristics, such as low fundamental frequency, low and narrow formant position, and above-average vocal tract length, which are reliable predictors of voice attractiveness, showed a positive association with mucosal immunoglobulin A (IgA) levels, whereby IgA levels were considered an indicator of physical condition (Arnocky et al., 2018). Still, the relationship between perceived attractiveness and immune quality requires further investigation because other studies delivered incompatible findings. Skrinda et al. (2014), for instance, reported no significant association between voice characteristics (formant frequency) and immune response measured by an elevation of antibodies against hepatitis B after vaccination. Also, some masculinity-related traits (Nowak-Kornicka et al., 2020) and body symmetry (Pawlowski et al., 2018) were in general not reflected in innate or adaptive immunity.

Nevertheless, cues to immune system functioning need not be restricted to visual or vocal ones. One might expect that odor-based cues could provide reliable markers of immune system functioning due to a more intimate association between the functioning of the immune system and body odor metabolism. It has been shown that the quality of body odor is affected by both metabolic conditions and infectious diseases. For example, odor samples collected a few hours after endotoxin injection were perceived as more aversive than samples from healthy donors. This effect was associated with the level of immune system activation: proinflammatory cytokines IL-6 and TNF- α mediated the effect on pleasantness and intensity of body odor (Olsson et al., 2014). Moreover, body odor from individuals injected with endotoxin had a negative effect on liking of facial images of both healthy and sick individuals (Sarolidou et al., 2020a). It is known that early cues of sickness may be detected even from urine: samples collected from individuals injected with endotoxin resulted in an increased urine odor averseness and volatile composition altered in comparison with urine samples from individuals injected with saline placebo (Gordon et al., 2018).

The expected association between attractiveness and responsiveness of the immune system might be mediated by levels of steroid hormones, specifically testosterone and cortisol. It has been proposed that testosterone has an immunosuppressive effect (Folstad and Karter, 1992). The suppressive effect of testosterone has been demonstrated in cell immunity, for example, in T-lymphocytes (Trigunaite et al., 2015). Testosterone also negatively influences lymphatic tissues (Weinstein et al., 1984) and inhibits the function of neutrophils (Marin et al., 2010). Still, empirical evidence in favor of the immunocompetence hypothesis is rather mixed (for a review, see Roberts et al., 2004 and Foo et al., 2017). The effect of the main stress-related hormone, cortisol, varies depending on the length of its production: while a sharp increase in stress hormone levels can boost the immune response to an antigen and lessen the impact of inflammation (Yeager et al., 2011), chronically elevated cortisol levels can weaken immune responsiveness and thus increase susceptibility to diseases (Kiecolt-Glaser and Glaser, 1995; Elenkov and Chrousos, 1999). The exact mechanism of the effect of testosterone and cortisol on the immune system remains unclear.

Steroid hormones, too, seem to influence body odor quality. It has been shown that body odor of men with higher testosterone levels is perceived as more attractive (Thornhill et al., 2013), and while another study did not replicate this finding, it found a positive association between cortisol levels and perceived odor attractiveness (Rantala et al., 2006).

Our main goal was to test the association between the functioning of human immune system and perceived body odor quality. In Study 1, we experimentally activated immune system by vaccinating volunteers against hepatitis A and B and meningococcus. Like previous studies, we used the levels of specific antibodies as a proxy for immune responsiveness. To stimulate the various components of the immune system, we employed vaccines against both viral (hepatitis) and bacterial (meningococcus) infections. We also measured testosterone and cortisol levels and body composition (Pawłowski et al., 2017) due to their possible immunomodulatory properties. In Study 2, we employed a larger sample and investigated an association between general immune functioning (without experimental stimulation) and perceived body odor health, intensity, and attractiveness. We used key markers of both innate and adaptive immunity to test the specificity of possible associations between body odor quality and immune system functioning.

2. Methods

Study 1 was conducted at the Charles University (Prague, Czech Republic). The study was approved by the Institutional Review Board of Charles University (approval number 20/2016) and its design preregistered prior to the data analyses (https://osf.io/69zgc/). Study 2 was carried out at the University of Wroclaw (Wroclaw, Poland). All its aspects were approved by the Institutional Review Board at the Institute of Psychology, University of Wrocław. All medical procedures involved in it were approved by the Bioethics Commission at the Lower Silesian Chamber of Physicians and Dentists' Ethics Committee (2/PB/2013) and conducted as part of a larger project by certified medical staff in a private clinic. In both studies, participants were informed about our goals and indicated their consent by signing an informed consent form. Study 1 was conducted as part of a larger project investigating possible associations between olfactory, visual, and vocal attractiveness and immunoreactivity. Immunological parameters of participants in Study 2 were also measured as part of a larger project that included other variables unrelated to the scope of the current study (for details, see Pawłowski et al., 2017 and Nowak et al., 2018).

2.1. Study 1

2.1.1. Body odor donors

Twenty-one Czech men aged 18–40 years (mean = 26.2; SD = 4.62) were recruited as body odor donors. Participation requirements were the following: good general health, non-smoking, not shaving one's armpits (Kohoutová et al., 2012), and not being vaccinated against hepatitis A/B or meningococcus in the past decade (e.g., Shepard et al., 2006). As compensation for their time and potential inconveniences, participants received 400 CZK (approx. €15) and the first dose of vaccines for free.

2.1.1.1. Body odor sampling procedure. Body odor samples were collected twice: once in the evening before vaccination and once 14 days after vaccination, when the highest antibody response was expected. All participants completed a questionnaire regarding their medical history and their health status was checked by a physician before vaccination. Subsequently, they were vaccinated against hepatitis A/B (Twinrix) and meningococcus (Menveo). Moreover, we obtained from participants three blood samples (5 ml each) to assess the levels of specific antibodies and steroid hormones (testosterone, cortisol). To determine the basal levels, we collected a blood sample before vaccination. The second sample was collected 14 days later together with the second body odor sample, and the last one 30 days post-vaccination (recommended interval for a second dose of hepatitis vaccine) to assess the dynamics of levels of specific antibodies. Vaccination was performed by a physician,

who also took the initial blood samples. Other blood samples were collected by phlebotomists at Prevedig laboratory, which also performed analyses of antibodies, steroid hormones, and CRP levels. To minimize potential variation in testosterone and cortisol levels due to circadian rhythms, all blood samples were collected at the same time of day (7 am) (Reinberg et al., 1978).

For body odor sampling, each participant received a list of instructions and a package containing plain cotton pads (approx. 9 × 7 cm; DM Ebelin, https://www.dm.cz/ebelin-odlicovaci-tampony-p4010355912930.html), 100% cotton white T-shirt (Adler Malfini Heavy; https://www.malfini.com/lv/en/product?category=t-shirts), non-perfumed soap (Balea ultra-sensitive, https://www.dm.de/balea-med-duschgel-ultra-sensitive-p4010355340412.html), and a surgical tape (Omnisilk 2.5 cm × 9.2 m; https://www.benu.cz/naplast-omnisilk-bile-hedvabi-2–5 cm×9–2 m-1ks). The day before and on the day of sampling, i.e., for about 48 h, participants were asked to avoid aromatic foods (e.g., spices, blue cheese, garlic), alcoholic beverages or other drugs, demanding physical activities (e.g., jogging), sex, and the use of fragranced products, which might all affect the quality of their body odor (Havlicek and Lenochova, 2006; Lenochova et al., 2009). Donors' conformity with these instructions was checked by a questionnaire completed when handing over the body odor samples (for the questionnaire, see Supplementary materials). On the night of sampling, donors washed their armpits using the non-perfumed soap and attached cotton pads to both armpits using a surgical tape. To avoid contamination by extrinsic ambient odors, they wore a 100% cotton T-shirt previously washed without any fragranced product as the first layer of clothing. They wore the cotton pads for 12 h overnight (this has been shown to be sufficient for body odor collection; cf. (Havlíček et al., 2011). 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 and kept there until the rating session to avoid further microbial activity that could alter the quality of the body odor (Lenochova et al., 2009).

2.1.2. Body odor raters

Eighty-eight Czech women aged 18–40 years (mean = 22.9; SD = 2.85) participated as body odor raters. To increase the sensitivity of detection of possible effects, we recruited only female raters. This is because on average, women achieve higher scores on various aspects of olfactory perception (for a review, see Brand and Millot, 2001) and they tend to place higher importance on body odor when choosing a potential partner (Havlicek et al., 2008). Participation criteria were good respiratory health and no use of hormonal contraception. Onset of most recent menstruation and usual length of menstrual cycle were recorded by a questionnaire. As a compensation for their time, they received 200 CZK (approx. €8).

2.1.2.1. Body odor rating procedure. Rating of body odor samples took place on two days (day 1: n = 43; day 2: n = 45) to accommodate the number of raters. It was conducted in a well-ventilated, quiet room with temperature of 18.2–20.7 °C (day 1) and 18.7–20.6 °C (day 2) and humidity 28–31% (day 1) and 27–28% (day 2). Body odor samples were presented in 500 ml opaque jars labelled by a code. Each sample was rated for its attractiveness, intensity, and healthiness on a 7-point scale (e.g., 1 – very unattractive, 7 – very attractive). For each session, odor samples were randomly split in three subsets and raters took breaks between each set to avoid sensory adaptation. Each rater thus participated in one session and rated 24 odor samples in total: 21 body odor samples (each donor before or after vaccination) and 3 controls (blank cotton pads). Body odor samples were randomly split in two sets (before vs. after vaccination) and within a particular rating session, each odor donor was included only once. Hence, in the first session, each rater was presented with a randomized mix of samples from before or after vaccination from all donors. In the second session, other raters were presented with samples from the same donors collected during the other condition (so that when, e.g., a donor was represented in the first session by a sample taken before vaccination, in the second session the donor was represented by post-vaccination sample). From each donor, we used body odor samples from a randomly selected armpit (either left or right) to avoid possible side-related perceptual differences (Ferdenzi et al., 2009). Samples were presented in a randomized order to avoid systematic bias within a rating session. After evaluation, raters completed an anonymous questionnaire regarding their demographic data (e.g., place of residence, education, occupation) and olfactory abilities (e.g., self-rated olfactory abilities, allergies, common cold).

2.1.3. Vaccine characteristics

To induce immune system response, we used the Twinrix Adult vaccine against hepatitis A/B and Menveo vaccine against meningococcus. Menveo is applied to prevent 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). Twinrix Adult 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). They might be applied together and are widely used in the Czech Republic. Both were applied intramuscularly, each in one arm.

2.1.4. Laboratory assays

All laboratory analyses were performed in a certified Prevedig laboratory in Prague (prevedig.cz). Levels of antibodies against hepatitis A (Anti-HAV) were measured from serum by Elecsys electrochemiluminescence immunoassay (Park and Kricka, 2013). This sensitive method measures antibodies or antigens in a sample: ruthenium chelate is added to label the substance of interest and provide light emission (Erler, 1998). This principle is used by fully automatized immunological analyzers. First, one incubates a sample in which anti-HAV binds itself to HAV antigen; then ruthenium chelate is added to occupy the remaining HAV-binding sites. The entire complex is captured on the surface of an electrode, where electric charge causes a chemiluminescent emission of photons. The results are read from calibration curves of the spectrophotometer.

Antibodies against hepatitis B (Anti-HBs) were measured from serum using the same method as described above for measuring antibodies against hepatitis A. The analysis of anti-HBs was included only for exploratory purposes because a relatively large proportion of donors either had high levels of antibodies at the baseline (N = 7) and was excluded from the further analyses or did not respond to vaccination (N = 5) and were included in the analyses. Participants may have been either unaware of some recent vaccination or nonresponsive to the vaccine: approx. 10% of population fails to respond to hepatitis B vaccine (Sjogren, 2005).

Antibodies against meningococcus (Anti-Mnk) were also examined from serum. They were analyzed by enzyme immunoassay (ELISA), one of the basic methods for determination of serum antibodies. In this method, an antigen on a special board reacts with antibodies present in participant's serum (primary antibodies). Afterwards, specially labeled secondary antibodies are added, which bind to the primary (participant's) antibodies with the antigen. A chromogenic substrate, added in the last phase, causes a color response that is measured by spectrophotometer (Belo et al., 2010). A response is considered sufficient if present in a ratio at least 1: 4 titres (serum dilution level where antibodies still react with antigens) but ideally in a higher ratio (Borrow et al., 2001).

Total testosterone was analyzed by chemiluminescence (CLIA), where energy is released by a reaction between testosterone, polyclonal anti-testosterone antibodies, and a tracer (Luppa et al., 1997). Hydrogen peroxide and either luminol or isoluminol are added to the mixture to react with the substrate. Peroxidase, here labeled by one of the immunoreactants, supports the formation of an amino phthalate dianion,

which emits light that is measured by a spectrophotometer (Kim et al., 1995).

Cortisol was analyzed by electrochemiluminescence. The method is similar to the HAV antibodies, but the sample was incubated with a specific anti-cortisol antibody labeled with ruthenium chelate.

2.1.5. Statistical analysis

An Intraclass Correlation (ICC) analysis using IBM SPSS Statistics v. 25 was performed to determine consistency among raters with two-ways mixed effect models for each group rating the same set of samples. We were mainly interested in single measures because they show the reliability of rating for one typical rater. Average measures show the reliability of different raters averaged across the sample. Other statistical tests were performed using Jamovi v. 0.9.6.9 software. To assess correlations between perceived odor attractiveness, intensity, healthiness (pre-vaccination), and differences in antibody levels (pre-vaccination and 30 days post-vaccination), we employed linear mixed-effects models (LMMs) using GAMLj jamovi module. In all three models, the rated characteristics (attractiveness, intensity, and healthiness of body odor) were entered as a dependent variable and differences in specific antibodies (Anti-HAV and Anti-Mnk) as fixed effect factors. To control for variability in donors' and raters' characteristics, we set donor and rater ID as a random effect. We used the variance of random components to estimate the contribution of each random effect to the variance of the dependent variable. Ex. of the used model script is: model_Attractiveness <- lmer(Attractiveness_odor ~ 1 + Anti-HAV + Anti-Mnk + (1|ID_rater) + (1|ID_donor)). For exploratory purposes, we also added anti-HBs levels into the mixed-effects models. The proportions of reduced error (pseudo R^2) for LMMs are reported in R^2 marginal (R^2_M) and R^2 conditional (R^2_C). Effect estimates of LMMs are stated with 95% confidence intervals [LL, UL].

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 body mass index (BMI), we employed a generalized linear model (GLM) using GAMLj jamovi module. In both models, specific antibodies (Anti-HAV and Anti-Mnk) were entered as dependent variables and steroid hormones and BMI as covariates. Ex. of the used model script is: Anti-HAV ~ 1 + basal cortisol + basal testosterone + BMI. To explore a possible relationship between family income (proxy for socioeconomic status), basal levels of steroid hormones, and elevations in specific antibodies (Anti-HAV, Anti-Mnk), we employed Spearman's correlation.

2.2. Study 2

2.2.1. Body odor donors

In Study 2, 35 participants (21 women and 14 men) aged 20–35 years (women: $M = 26.7$; $SD = 4.77$; men: $M = 26.6$; $SD = 5.16$) took part as body odor donors. They received a small gift (a set of cosmetics) as a reward for their time and effort. Criteria for participation were general good health (no chronic diseases or infections) and no use of medications.

2.2.1.1. Body odor sampling procedure. On the day of measurement of general immune system parameters, body odor donors received a package with two 100% cotton pads (approx. 7×10 cm), a non-perfumed soap, hypoallergenic surgical tape, three zip-lock plastic bags, and a new 100% cotton T-shirt (Fruit of the Loom; https://www.fruitoftheloom.eu/shop/fruit-underwear-t-3-pack/). They were asked to collect the odor sample within the following week. Donors were also instructed to avoid using scented cosmetics or foods and drinks that could influence their body odor (e.g., alcohol, garlic) starting on the day before odor collection. They were asked to wash their bodies with the non-perfumed soap, attach the cotton pads to their armpits with the surgical tape and wear the T-shirt provided by us to avoid contamination by extrinsic ambient odors. They wore the cotton pads for following 12 h during the night (cf. (Havlíček et al., 2011), placed them in the zip-log bags, and returned them to the experimenters. For more details on the body odor sampling procedure, see Sorokowska et al., (2016). Collected samples were frozen at −20 °C until the day of body odor rating (Lenochova, Roberts and Havlíček, 2009). A detailed description of general immune system parameters analyzed in this study, as well as methods used for their measurement, are described below in the following chapter (2.2.3.) and in the supplementary information of Nowak et al. (2018).

2.2.2. Body odor raters

A group of 95 raters (49 women, 46 men) aged 19–43 years (women: M = 21.2; SD = 1.79; men: M = 22.6; SD = 4.80) participated as body odor raters in Study 2. Prior to the assessments, they completed a questionnaire on diseases or olfactory impairments that might potentially affect their odor perception and female raters also recorded their status regarding the use of hormonal contraception and the date of their last period. Only one female rater reported using hormonal contraception. No participants reported any health problems. As a compensation for participation in the rating session, odor raters received some sweets.

2.2.2.1. Body odor rating procedure. The rating was divided in nine sessions, each comprising four or five randomly selected odor samples that contained at least one male and one female sample. For each session, experimenters recruited between nine and twelve participants with approximately equal representation of males and females. The raters completed the survey mostly individually but occasionally, they were also allowed to enter in the testing room in pairs, e.g., in case two friends both agreed to participate in the study when approached by an experimenter. The rating session was, however, performed in an adjusted testing room that enabled the participants to sit separately and assess the samples presented by the experimenter individually.

The rating took place in a quiet, well-ventilated room. Body odor samples were presented in closed, non-transparent, 0.5 liter brown glass jars labelled with letters. Participants were asked to open each jar, smell the body odor sample, imagine a person who donated it, and rate the attractiveness, intensity, and healthiness of a body odor sample on provided scoring sheets. The ratings were performed on 0–10 Likert-type scales (e.g., 0 – not healthy at all to 10 – very healthy). As in Study 1, the samples were presented in a random order.

2.2.3. Immunological parameters and laboratory assays

To evaluate the general quality of odor donors' immune system, we measured the key parameters/mechanisms reflecting their innate and adaptive immunity. Of innate immunity mechanisms, we measured the parameters that constitute humoral (complement system, antibacterial enzymes) and cellular (neutrophil functions) innate immunity. Of adaptive immunity mechanisms, we measured the level of main effectors cells (lymphocytes) and molecules (immunoglobulins).

As part of the humoral innate immune system, we measured both complement and lysozyme activity. The complement system consists of approx. 30 soluble components present in various body fluids which are sequentially activated in response to infection. The complement cascade leads, for instance, to a damage of pathogen's membrane by a membrane attack complex (SC5b-9), formed during this process. It can cause lysis of pathogen cells and is particularly effective against Gram-negative bacteria. Total complement activity was measured via the concentration of Terminal Complement Complex (SC5b-9 complex) in zymosan-activated serum samples using a commercial EIA kit (MicroVue™ SC5b-9, QUIDEL®). The results are expressed in ng/ml. A higher concentration of SC5b-9 indicates higher immune activity.

Lysozyme is the most abundant antimicrobial enzyme in the human serum, with strong activity on Gram-positive bacteria in which it causes cell lysis. Antibacterial activity of the serum in lysis of *Micrococcus*

lysodeicticus was measured using a turbidimetric assay. The results are presented as the difference in absorbance values between control and test samples, whereby a greater difference reflects higher lysozyme activity in the tested serum.

As a marker of innate cellular immunity, we used neutrophil functions. Neutrophils are the first cells recruited to infection site and a key constituent of innate immunity. They are the main fraction of professional phagocytes in the blood that can engulf and kill pathogens by various intracellular mechanisms, including the production of reactive oxygen species (oxidative burst). Quantitative analysis of phagocytosis by neutrophils was conducted using commercial kits (Phagotest™, Glycotope) and measured by flow cytometry (FACS Calibur flow cytometer, BD®). The results are expressed as mean fluorescent intensity of phagocytosing neutrophils. Neutrophils' ability to produce reactive oxygen species after stimulation was measured by chemiluminescence methods. The results are expressed as the mean area under chemiluminescence curve (AUC_{CL}) for the stimulated test sample, divided by AUC_{CL} for control.

As markers of the adaptive immune system, we used total lymphocyte T and B counts. Lymphocytes are the main effectors of adaptive immunity. T and B cells are the key lymphocyte populations engaged in adaptive cellular and humoral immune response, respectively. B cells differentiate into plasma cells and secrete immunoglobulins, whereas the T-cells, depending on the subpopulation, are responsible for elimination of infected cells (CD8 cytotoxic T-cells) or stimulation of antibody- or cell-mediated immune response (CD4, helper T-cell). T-cell subpopulations (CD4/CD8 ratio) also play an important role in immune homeostasis. Low levels of CD4/CD8 are a sign of chronic immune response associated with, e.g., a viral infection (Amadori et al., 1995). Immunophenotyping of blood lymphocytes (quantitative analysis of lymphocytes and their subpopulation) was conducted by commercial kits (TriTest, BD®), measured by flow cytometry (FACS Calibur flow cytometer, BD®), and processed by BD CellQuest software to calculate the number of cells per microliter of blood (cells/μl). The CD4/CD8 ratio was calculated by dividing the CD4 count by the CD8 count.

Total immunoglobulin levels were used as a marker of adaptive humoral immunity because they provide key information on humoral adaptive immune status. In human serum, IgG is the most common class of antibodies capable of binding to antigens with high specificity. Such antigen-antibody complexes are then effectively eliminated by various innate and/or adaptive immune mechanisms. Total levels of immunoglobulins were measured using enzyme-linked immunosorbent assay according to a calibrated procedure described in Orczyk-Pawiłowicz et al. (2013). Total IgG antibody concentrations were expressed in g/L.

2.2.4. Statistical analyses

An ICC analysis using IBM SPSS Statistics v. 25 was performed to determine consistency among raters with two-ways mixed effect models for each group rating the same set of samples. Other statistical tests were performed using jamovi v. 1.6.6.0 software. To test immune system predictors of body odor quality, we employed LMMs using GAMLj jamovi module again. In total, we ran six separate models – humoral innate and adaptive immune system separately and one model for each rated characteristic – using the REML fit. In all models, the rated characteristic (e.g., intensity) was a dependent variable, while odor donor ID and rater ID were set as random-effects factors (again, we used the variance of random components to estimate the contribution of each random effect to the variance of the dependent variable), so that for instance the model for body odor intensity was model_Intensity <- lmer (Intensity_odor ~ 1 + IgG + CD4/CD8ratio + CD3 + CD19 + (1 | ID_donor)+ (1 | ID_rater)). The proportions of reduced error (pseudo R^2) for LMMs are reported in R^2 marginal (R^2_M) and R^2 conditional (R^2_C). Effect estimates of LMMs are stated with 95% confidence intervals [LL, UL].

3. Results

3.1. Study 1

Descriptive statistics for analyzed parameters, such as ratings of body odor quality, specific antibody levels, steroid hormone levels, BMI and monthly family income are presented in Table 1.

Inter-rater agreement varied from rather low to moderate depending on the rating session; for more details, see Supplementary materials.

3.1.1. Relationship between reactivity of the immune system and perceived body odor quality

A linear mixed-effect model shows that neither perceived pre-vaccination body odor attractiveness ($R^2_C = 0.333$, $R^2_M = 0.005$), intensity ($R^2_C = 0.445$, $R^2_M = 0.025$), nor healthiness ($R^2_C = 0.249$, $R^2_M = 0.005$) were predicted by increase in the levels of specific antibodies; for details, see Table 2. In the model with included Anti-HBs, healthiness ($R^2_C = 0.209$, $R^2_M = 0.034$) was negatively predicted by increase in Anti-HBs but the effect was negligible ($\beta < -0.001$); for more details, see Table S4 in Supplementary materials.

3.1.2. Relationship between the elevations of levels of specific antibodies, basal steroid hormone levels, and BMI

Neither basal levels of testosterone, cortisol, nor the BMI predicted elevations in specific antibodies Anti-HAV ($R^2 = 0.097$, $R^2_{adj} = -0.061$) and Anti-Mnk ($R^2 = 0.094$, $R^2_{adj} = -0.065$). For detailed results, see Table 3. Moreover, neither basal levels of testosterone ($\rho = -0.282$, $p = 0.215$), nor cortisol ($\rho = 0.133$, $p = 0.565$), Anti-HAV ($\rho = -0.111$, $p = 0.632$), Anti-HBs ($\rho = 0.008$, $p = 0.978$), or Anti-Mnk ($\rho = 0.212$, $p = 0.356$) were significantly correlated with family income.

3.2. Study 2

Table 4 provides descriptive statistics for ratings of body odor quality and both innate and adaptive immune system parameters.

Again, inter-rater agreement varied from low to excellent depending on the rating session. For more details, see Table S3 in Supplementary materials.

3.2.1. The relationship between the innate immune system and perceived body odor quality

None of the innate immune system measures predicted perceived body odor attractiveness ($R^2_C = 0.466$, $R^2_M = 0.012$) or intensity ($R^2_C = 0.333$, $R^2_M = 0.024$). Body odor healthiness ($R^2_C = 0.331$, $R^2_M = 0.05$) was negatively statistically significantly predicted by complement activity, but the effect size was negligible ($\beta < -0.001$). For detailed

Table 1
Descriptive statistics for ratings of body odor quality before vaccination, specific antibodies (the difference between 30 days after vs. before vaccination state), testosterone and cortisol basal levels, BMI, the amount of adipose tissue and family income (N = 21; for anti-HBs N = 14). The mean (SD) for body odor ratings was calculated as a mean from aggregated ratings for each body odor donor. Values denoted by * show mean minimum and mean maximum ratings of samples.

Parameter name	Mean	SD	Range (min – max)
Body odor attractiveness	3.34	0.76	1.59–4.86*
Body odor intensity	4.38	1.1	3.06–6.62*
Body odor healthiness	3.99	0.63	2.55–5.12*
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
Anti-HBs antibodies (IU/I)	544	449	0–997
Basal testosterone (ug/l)	4.33	1.23	2.25–7.1
Basal cortisol (nmol/l)	471	91.4	282–662
BMI	24.2	4.69	18.7–39.5
Adipose tissue (%)	17.5	6.86	5–32.8
Family income (in thousands of CZK)	34.9	17.8	15–67.5

Table 2
Relationship between reactivity of the immune system and perceived body odor quality. Variance of random components = VRC. Attractiveness ratings: for odor donor ID, VRC = 0.584, SD = 0.764, ICC = 0.234, for odor rater ID, VRC = 0.355, SD = 0.596, ICC = 0.157. Intensity ratings: for odor donor ID, VRC = 1.217, SD = 1.103, ICC = 0.38, for odor rater ID, VRC = 0.286, SD = 0.535, ICC = 0.126. Healthiness ratings: for odor donor ID, VRC = 0.378, SD = 0.615, ICC = 0.151, for odor rater ID, VRC = 0.309, SD = 0.556, ICC = 0.127.

Characteristic	Parameters	F	β	95% CI (LL, UL)	df	t	SE	p
Attractiveness	Anti-HAV	0.225	0.084	-0.263, 0.431	18.1	0.586	0.177	0.641
	Anti-Mnk	0.197	-0.004	-0.024, 0.016	18.1	-0.443	0.01	0.663
Intensity	Anti-HAV	0.052	-0.057	-0.549, 0.434	18.1	-0.229	0.251	0.822
	Anti-Mnk	1.331	0.017	-0.012, 0.045	18.1	1.154	0.015	0.264
Healthiness	Anti-HAV	0.401	0.093	-0.194, 0.379	17.9	0.634	0.146	0.534
	Anti-Mnk	0.265	-0.004	-0.021, 0.012	17.9	-0.515	0.008	0.613

Table 3
Relationship between reactivity of the immune system, basal steroid hormone levels, and BMI. Ex. of the used model script is: Anti-HAV ~ 1 + basal cortisol + basal testosterone + BMI.

Characteristic	Parameters	F	β	95% CI (LL, UL)	df	t	SE	p
Anti-HAV	Cortisol	1.809	0.352	-0.002, 0.009	17	1.345	0.003	0.196
	Testosterone	0.886	0.045	-0.494, 0.568	17	0.146	0.252	0.886
	BMI	0.356	0.197	-0.107, 0192	17	0.596	0.042	0.559
Anti-Mnk	Cortisol	0.91	-0.25	-0.153, 0.058	17	-0.954	0.049	0.353
	Testosterone	0.869	0.286	-5.118, 13.228	17	0.933	4.348	0.364
	BMI	0.014	0.039	-2.435, 2.728	17	0.12	0.122	0.906

Table 4
Descriptive statistics including the mean, SD, and range of the target sample for body odor quality ratings, innate (complement activity, lysozyme, activity, phagocytosis, oxidative burst) and adaptive (IgG, CD4/CD8 ratio, CD3, CD19) immune system parameters (N = 36). Values denoted by * show the mean minimum and mean maximum ratings of samples.

	Parameter name	Mean	SD	Range
Body odor quality	Body odor attractiveness	3.47	2.66	0.9; 6.286*
	Body odor intensity	5.63	2.8	1.778; 8.8*
	Body odor healthiness	4.84	2.58	2.3; 7.444*
Innate immune system	Complement activity (ng/ml)	204336	66863	62337; 311097
	Lysozyme activity	0.389	0.0805	0.23; 0.59
	Phagocytosis	185	74.9	104; 386
	Oxidative burst	7.14	5.25	2.35; 28.5
Adaptive immune system	IgG (g/l)	10.5	3.12	4.54; 19.2
	CD4/CD8 ratio	1.78	0.62	0.53; 3.34
	CD3 (cells/μl)	1650	643	712; 3047
	CD19 (cells/μl)	229	149	52.5; 640

results, see Table 5. We found sex differences in phagocytosis levels and therefore entered the sex of odor donor in the analysis, but the results were similar. For more details, see Table S1 in Supplementary materials.

3.2.2. Relationship between the adaptive immune system and perceived body odor quality

None of the adaptive immune system measures predicted perceived body odor attractiveness ($R^2_C = 0.48$, $R^2_M = 0.021$), intensity ($R^2_C = 0.352$, $R^2_M = 0.02$) or healthiness ($R^2_C = 0.323$, $R^2_M = 0.035$); for detailed results, see Table 6. We found a sex difference in CD4/CD8 ratio levels and therefore entered donor's sex in the analysis. The results showed that the CD4/CD8 ratio positively predicted body odor intensity (β = 1.507); for more details, see Table S2 in Supplementary materials.

4. Discussion

The main aim of the two studies was to test a possible association between functioning of the immune system and perceived body odor quality in humans. Contrary to our predictions, neither of the studies showed a significant relationship between immune system functioning and body odor quality. In Study 1, elevation of antibodies against hepatitis A (Anti-HAV) and meningococcus (Anti-Mnk) after vaccination

Table 5
Innate immune system predictors of body odor quality. Variance of random component = VRC. Attractiveness rating: for odor donor ID, VRC = 2.43, SD = 1.56, ICC = 0.368; for odor rater ID, VRC = 1.11, SD = 1.05, ICC = 0.211. Healthiness rating: for odor donor ID, VRC = 1.221, SD = 1.105, ICC = 0.207; for odor rater ID, VRC = 0.747, SD = 0.864, ICC = 0.138. Intensity rating: for odor donor ID, VRC = 2.416, SD = 1.554, ICC = 0.306; for odor rater ID, VRC = 0.113, SD = 0.337, ICC = 0.02.

Characteristic	Parameters	F	β	SE	95% CI (LL, UL)	df	t	p
Attractiveness	Complement activity	0.582	< -0.001	< 0.001	< -0.001, < 0.001	29.4	-0.763	0.451
	Lysozyme activity	0.683	3.124	3.779	-4.282, 10.529	27.6	0.827	0.416
	Phagocytosis	< 0.001	< 0.001	0.004	-0.008, 0.008	29	0.008	0.994
	Oxidative burst	0.052	0.013	0.057	-0.098, 0.124	28.5	0.228	0.821
Healthiness	Complement activity	5.7	< -0.001	< 0.001	< -0.001, < -0.001	30.5	-2.387	0.023
	Lysozyme activity	1.264	3.279	2.916	-2.437, 8.995	27.8	1.124	0.271
	Phagocytosis	0.017	< 0.001	0.003	-0.006, 0.007	30.4	0.130	0.897
	Oxidative burst	0.036	0.008	0.044	-0.078, 0.095	29.8	0.189	0.851
Intensity	Complement activity	0.015	< -0.001	< 0.001	< -0.001, < 0.001	28.8	-0.124	0.902
	Lysozyme activity	1.276	-4.323	3.827	-11.825, 3.178	28.1	-1.130	0.268
	Phagocytosis	0.318	0.002	0.004	-0.006, 0.011	29.6	0.564	0.577
	Oxidative burst	0.388	-0.036	0.057	-0.148, 0.077	29.1	-0.623	0.538

Table 6
Adaptive immune system predictors of body odor quality. Variance of random component = VRC. Attractiveness rating: for odor donor ID, VRC = 2.04, SD = 1.43, ICC = 0.341; for odor rater ID, VRC = 1.15, SD = 1.07, ICC = 0.225. Healthiness rating: for odor donor ID, VRC = 1.092, SD = 1.045, ICC = 0.189; for odor rater ID, VRC = 0.827, SD = 0.909, ICC = 0.150. Intensity rating: for odor donor ID, VRC = 2.213, SD = 1.487, ICC = 0.289; for odor rater ID, VRC = 0.053, SD = 0.231, ICC = 0.01.

Characteristic	Parameters	F	β	SE	95% CI (LL, UL)	df	t	p
Attractiveness	IgG	0.166	-0.039	0.096	-0.227, 0.149	28.9	-0.408	0.686
	CD4/CD8 ratio	0.167	-0.065	0.502	-1.049, 0.918	27.2	-0.13	0.898
	CD3	1.301	< 0.001	< 0.001	< -0.001, 0.002	28.8	1.141	0.263
	CD19	1.617	-0.004	0.003	-0.01, 0.002	27.8	-1.271	0.214
Healthiness	IgG	0.021	-0.011	0.075	-0.159, 0.137	30.6	-0.145	0.886
	CD4/CD8 ratio	0.011	0.041	0.393	-0.728, 0.811	28.6	0.105	0.917
	CD3	1.833	< 0.001	< 0.001	< -0.001, 0.002	31.3	1.354	0.186
	CD19	4.187	-0.005	0.002	-0.01-, < 0.001	29	-2.046	0.05
Intensity	IgG	0.017	-0.013	0.103	-0.216, 0.189	27.9	-0.13	0.898
	CD4/CD8 ratio	0.053	0.126	0.547	-0.945, 1.197	27.7	0.23	0.82
	CD3	5.97e-4	< 0.001	< 0.001	-0.001, 0.002	28.5	0.024	0.981
	CD19	0.616	0.003	0.003	-0.004, 0.009	27.4	0.785	0.439

predicted neither body odor attractiveness, nor intensity, nor healthiness. There was also no significant relationship between the testosterone and cortisol levels and levels of Anti-HAV and Anti-Mnk antibodies. Moreover, BMI had no significant effect on the levels of specific antibodies. In Study 2, we moreover found that body odor qualities were likewise not associated with markers of innate or adaptive immune system.

Our research assessed possible links between body odor quality and immunocompetence defined as disease resistance and ability to adequately react to an antigen. As mentioned in the introduction, individuals with well-functioning immune system should be favored in mate choice due to both direct and indirect fitness-related benefits, such as increased parental care by more immunocompetent mates and lower susceptibility to infections in future offspring (Møller, 1995). Previous studies reported an association between male facial attractiveness and reactivity of the immune system measured by an increase in cytokines after administration of the LPS (Phalane et al., 2017) or an increase in antibodies after vaccination against hepatitis B in men (Rantala et al., 2012), though not in women (Rantala et al., 2013).

In the exploratory part of the study, we added into the statistic model differences in levels of Anti-HBs as another fixed effect factor, but we had to exclude donors with high levels of antibodies at the baseline (N = 7) from the analysis. Anti-HBs statistically significantly predicted body odor healthiness but the effect size was negligible. Still, due to a small sample size these results should be interpreted with caution.

So far, studies on health-related olfactory cues focused primarily on current health status and did not specifically test possible associations between odor cues and immunocompetence, nor did they focus on the mating context. For example, body odor samples from individuals infected with *Neisseria gonorrhoeae* were rated as less pleasant and described as more putrid than samples from healthy persons (Moshkin et al., 2012). A study by Sarolidou et al. (2020b) showed that body odor samples from individuals with naturally occurring respiratory infection were nominally (but not significantly) rated as more intense, more disgusting, and less pleasant and healthy than samples from the same participants when healthy. These studies are in line with findings on aversive body odor qualities of infected conspecifics in various rodent species (e.g., Kavaliers and Colwell, 1995; Zala et al., 2004). Furthermore, odor samples collected from men injected with LPS were perceived as more aversive even just several hours after injection (Olsson et al., 2014) and urine samples resulted in increased urine odor averseness, because the natural decrease of averseness in healthy urine was disrupted by the LPS (Gordon et al., 2018). While these behavioral studies are convincing, the specific mechanisms of body odor alterations during infections remain largely unknown. There are several possible pathways, including volatile chemicals derived either from the pathogen itself or related to activation of the immune system, or a combination of the two.

In contrast to the frequently investigated effect of health status, association between body odor and the effectiveness of immune system functioning in healthy individuals has not been directly tested in mammals yet. Interestingly, there is some evidence showing that in males of the mealworm beetle (*Tenebrio molitor*), attractiveness of their pheromones is positively associated with their immunocompetence measured by phenoloxidase activity and encapsulation (Rantala et al., 2003a, 2003b).

Our two studies showed that these results cannot be generalized to vertebrates. We focused on several aspects of immunoreactivity, namely response to vaccines (Study 1) and general immune qualities (Study 2). Neither of our studies indicated a significant relationship between these parameters and body odor attractiveness. It should be noted that the immunoreactivity we investigated in Study 1 might serve as a marker of an adequate reaction to pathogenic threat, although higher immune responsiveness does not necessarily amount to ideal disease resistance. If immune response is too strong (hypersensitivity) or inappropriate (autoimmunity), it negatively affects the viability and consequently also the fitness of the individual concerned. Besides, suppression of immune responses can in some circumstances be adaptive, for instance, when the risk of higher costs is more significant when arming the immune defense than the expected costs associated with infection (Hanssen et al., 2004). The underlying interplay between the immune system and impact on fitness cannot be explained by humoral immune response alone and to focus solely on antibodies levels is overly simplifying. Future studies may therefore include other immune components, such as cytokine response levels (Phalane et al., 2017).

Our finding of no significant effects in Study 2 suggests that the general – in healthy individuals both adaptive and innate – immunological parameters may be not easily perceptible in olfaction-mediated mate choice. An abovementioned study on the effect of LPS (Olsson et al., 2014) does, however, indicate that some aspects of the innate immune system might be reflected in human body odor. This supports a hypothesis that what affects odor qualities are not the “baseline” parameters of healthy organism we measured in our research but rather some infection-generated processes activated in response to a threat. Such putative mechanism would involve an interplay within skin microbiota which contributes to body odor production and immunity. There is, for instance, a well-documented relationship between the innate immune system and intestinal microbiota. Microbial metabolites, such as short-chain fatty acids, serve as messengers between intestinal microbiota and the immune system and can modulate immune response, especially of the inflammatory kind (Levy et al., 2017). But activation of the immune system also leads to the production of various metabolites and this mechanism need not be restricted to intestinal microbiota: it could affect the skin microbiota as well. The exact mechanism of excretion of specific metabolites in sweat is mostly unknown but we assume that it might affect body odor quality and be perceptible by conspecifics. It is also worth noting that local skin immunity might be more related to body odor than systemic immunity due to a direct

relationship between body odor, skin microbiota composition, and immunity mechanisms modulated by skin microbiota (e.g., (Belkaid and Segre, 2014)). All in all, there seems to be emerging body of evidence to the effect that body odor may serve as an effective cue to actual health status. Evidence regarding body odor as a marker of immunocompetence is, on the other hand, limited.

The inter-rater agreement in our study varied from relatively low to quite high values depending on the specific sessions. One may also argue that lack of significant association between body odor quality and immune system functioning could be due to insufficient inter-rater agreement. Such low agreement would suggest that ratings of body odor attractiveness are driven primarily by cues of genetic compatibility such as major histocompatibility complex, but available evidence is rather ambivalent (Havlíček et al., 2020).

Steroid hormones might serve as mediators between functioning of the immune system and body odor because they have an effect on both. Some studies show that immune response to antigens triggers a decrease in testosterone, which might suggest preferential use of energy and point to testosterone's immunosuppressive effect (Folstad and Karter, 1992). On the other hand, a meta-analysis targeting this issue showed no significant effect of testosterone on immune system function (Roberts et al., 2004; Foo et al., 2017). Besides, there is evidence that increased cortisol levels associated with acute stress may serve as a preparation for possible infection and may support the immune system following its activation (Elenkov and Chrousos, 1999; Yeager et al., 2011). In Study 1, we found no significant relationship between immunoreactivity (antibody levels) and steroid hormones. This is in line with the null results of a study that tested the effect of total testosterone on the efficiency of influenza vaccine (Nowak et al., 2018). Nevertheless, other in vitro studies found a remarkable immunosuppressive effect of testosterone on the level of antibody production (Kanda et al., 1996, 1997) and some correlational studies even found a positive association between total testosterone and Anti-Hbs antibodies after vaccination to hepatitis B (Rantala et al., 2012) or between free testosterone and the strength of response to influenza vaccine (Nowak et al., 2018). Furthermore, the association between testosterone levels and strength of immune response to vaccine might be mediated by socioeconomic status. Luoto et al. (2021) recently reported that Latvian men with a higher family income have a stronger immune response to vaccination against hepatitis B and their testosterone levels are likewise higher. Moreover, cortisol levels may be also negatively associated with family income, which is additionaly positively correlated with the strength of immune response to vaccine against hepatitis B in women (Rubika et al., 2020). Variation in socioeconomic status in our sample was relatively low, which may be why we did not observe any such association. Clearly, further studies are needed to disentangle the complex relationship between testosterone levels and functioning of the immune system.

Moreover, studies testing possible links between steroid hormones and body odor show an inconsistent pattern. In one study, cortisol did not predict body odor attractiveness in men (Thornhill et al., 2013), another study found a positive association between cortisol levels and perceived odor attractiveness (Rantala et al., 2006), and Butovskaya et al. (2013) even reported a negative relationship between cortisol and body odor attractiveness. Similar inconsistencies have been reported for testosterone: Thornhill et al. (2013) found a positive association between body odor attractiveness and testosterone levels, while Rantala et al. (2006) found no relationship. Thus, once again, based on available data one cannot at this point draw any meaningful conclusions about the relationship between steroid hormones and qualities of body odor in humans.

We also found no effect of BMI on the level of antibody production, although adipose tissue is believed to have immunomodulatory properties (Pawłowski et al., 2017). Obesity is related to various immune dysfunctions and impaired immune response. Obese individuals suffer from higher infection rates, worse wound healing, changes in leukocyte counts, and reduced antibody production (Marti et al., 2001). It should be noted, however, that most of our body odor donors had BMI within the normal range; only two individuals had BMI over 30, which is considered obesity. Any negative effect of increased amounts of adipose tissue on the production of antibodies might thus have been restricted to these individuals and might not show in average body weight variation, although Pawłowski et al. (2017) did find a negative effect of higher BMI on immune system functioning in average body weight variation.

4.1. Limitations

One limitation of Study 1 is the low sample size of body odor donors, although it is comparable with previous studies on body odor quality (see, e.g., Moshkin et al., 2012; Regenbogen et al., 2017). We faced considerable difficulties in recruiting participants because people hesitated to participate in a 'vaccination' study due to the recent anti-vaccination movement. Some volunteers had been vaccinated, mainly against hepatitis B, in the past ten years and had to be excluded from the study. Moreover, 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). In our sample, several participants showed some level of antibodies against hepatitis B already at the baseline measurement, while others either did not respond to vaccination or their antibody levels exceeded sufficient protective levels. In these individuals, we could not record exact antibody level values that could be entered into further analyses. For these reasons, antibodies against hepatitis B were included only for exploratory purposes. In future studies, we recommend using less common vaccines in order to facilitate recruitment of naive participants. Similar, to most body odor studies, we recruited only female raters to Study 1, because they tend to place higher importance on body odor when choosing romantic partners (Havlicek et al., 2008). In Study 2, we included body odor donors and raters of both sexes. However, inclusion of female participants generated menstrual cycle-related issues. There is robust evidence that perceived qualities of female body odor fluctuates across menstrual cycle with enhanced attractiveness around ovulation (Kuukasjärvi et al., 2004; Havlíček et al., 2006). Further, menstrual cycle-related changes were also observed in olfactory perception (Nováková et al., 2014) and variation in cycle phase might thus introduced further noise in data from female raters. Although we recorded the day of the cycle of the female donors included in Study 2, counting methods are notoriously unreliable (Gangestad et al., 2016), and we have, therefore, decided not to explore these data. Nevertheless, we suggest that future studies on adaptive and innate immunity and body odor should control this factor and explore the effects reported in our studies in larger samples of both male and female donors and raters to address possible sex differences in the observed effects and any potential, hormonal confounders.

5. Conclusion

To the best of our knowledge, this is the first systematic investigation of possible associations between directly measured functioning of the immune system and body odor quality. As markers of immunoreactivity, we measured the elevation of specific antibodies after vaccination, while functioning of the immune system was measured by key components of innate and adaptive immunity in healthy individuals. We found no significant association between activation of the immune system or its markers and the quality of body odor. Nevertheless, there is robust evidence to the effect that body odor quality is sensitive to current health status, especially to nonspecific antigens targeted primarily by the innate immune system. Therefore, we propose that future studies should focus on associations between body odor quality and changes in the activity or levels of components of innate immunity in response to immune stimulation.

Declarations of interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105363.

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Statement of contribution

This is to confirm that Mgr. Dagmar Schwambergová significantly contributed to the following publication:

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She contributed to the conceptualisation, investigation, methodology, data analysis, visualisation, manuscript writing and subsequent editing and revisions.

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

_____

doc. Mgr. Jan Havlíček, Ph.D.
supervisor

Chapter 13

THE CONTEXT MATTERS: WOMEN'S EXPERIENCES OF THEIR PARTNER'S ODOR IN INTIMATE AND SEXUAL ENCOUNTERS

Archives of Sexual Behavior

The context matters: Women's experiences of their partner's odor in intimate and sexual encounters

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Corresponding Author:	Anna Fišerová, Mgr. Charles University Faculty of Science: Univerzita Karlova Prirodovedecka fakulta Prague, CZECH REPUBLIC
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Charles University Faculty of Science: Univerzita Karlova Prirodovedecka fakulta
Corresponding Author's Secondary Institution:	
First Author:	Anna Fišerová, Mgr.
First Author Secondary Information:	
Order of Authors:	Anna Fišerová, Mgr.
	Žaneta Pátková
	Dagmar Schwambergová
	Lucie Jelínková
	Jan Havlíček
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Abstract:	Research shows that male body odor plays an important role in women's mate choice and that olfactory abilities are associated with women's sexual functioning. What remains unclear is what types of partner's odor actually shape the experience during intimate activities. This study therefore explored women's experience associated with the partner's various odors and investigated how they affect their intimate and sexual encounters. We performed semi-structured individual interviews with 20 single women and 20 women in a long-term relationship. Thematic analysis revealed four key natural odor types of the partner: body odor, sweat, genital odor, and semen odor. Further, we have identified three main types of fragrance odor (cologne, shower gel, and laundry agents) and investigated their perception in both intimate (hugging, kissing, cuddling, lying side by side) and sexual (intercourse, oral sex, ejaculation) contexts. Both partner's natural odor and fragrance affected women's emotional state (ranging from pleasant to unpleasant) and behavioral response (ranging from approach to avoidance of partner). Women's odor perception was frequently context-dependent, so that even mostly negatively perceived odors (e.g., semen, genital odor) were often accepted as part of the sexual encounter. Finally, women's perception was negatively modified by partner's specific sweat (after workday, work-out, or when the partner is ill) during intimate encounters. The results of the study highlight the complexity and inter-individual variability of the perception of partner's odor.

Cover Page

The context matters: Women's experiences of their partner's odor in intimate and sexual encounters

Anna Fišerová*, Žaneta Pátková, Dagmar Schwambergová, Lucie Jelínková, Jan Havlíček

Faculty of Science, Charles University, Prague, Czech Republic.

*Corresponding author:

Anna Fišerová: Faculty of Science, Charles University, Viničná 7, Prague, 128 00, Czech Republic.

E-mail: a.fiserova@natur.cuni.cz

ORCID IDs:

Anna Fišerová: https://orcid.org/0000-0002-5320-6550

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Declarations

Authors' Contributions

Anna Fišerová, Žaneta Pátková, Dagmar Schwambergová, Lucie Jelínková, and Jan Havlíček developed the study concept and design. Anna Fišerová performed data collection and data curation. Anna Fišerová and Žaneta Pátková conducted data analysis while consulting it with Dagmar Schwambergová, Lucie Jelínková, and Jan Havlíček. The first draft of the manuscript was written by Anna Fišerová and Dagmar Schwambergová. All authors commented on earlier versions of the manuscript and read and approved the final manuscript.

Competing Interest Statement

The authors are not aware of any competing interests.

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Ethics approval

The study was approved by the Institutional Review Board of the Charles University, Faculty of Science (approval number 21/10).

ABSTRACT

Research shows that male body odor plays an important role in women's mate choice and that olfactory abilities are associated with women's sexual functioning. What remains unclear is what types of partner's odor actually shape the experience during intimate activities. This study therefore explored women's experience associated with the partner's various odors and investigated how they affect their intimate and sexual encounters. We performed semi-structured individual interviews with 20 single women and 20 women in a long-term relationship. Thematic analysis revealed four key natural odor types of the partner: body odor, sweat, genital odor, and semen odor. Further, we have identified three main types of fragrance odor (cologne, shower gel, and laundry agents) and investigated their perception in both intimate (hugging, kissing, cuddling, lying side by side) and sexual (intercourse, oral sex, ejaculation) contexts. Both partner's natural odor and fragrance affected women's emotional state (ranging from pleasant to unpleasant) and behavioral response (ranging from approach to avoidance of partner). Women's odor perception was frequently context-dependent, so that even mostly negatively perceived odors (e.g., semen, genital odor) were often accepted as part of the sexual encounter. Finally, women's perception was negatively modified by partner's specific sweat (after workday, work-out, or when the partner is ill) during intimate encounters. The results of the study highlight the complexity and interindividual variability of the perception of partner's odor.

Keywords: Body odor; Fragrance; Romantic relationship; Sex; Olfaction; Chemical communication

INTRODUCTION

Human body odor plays a crucial role in various social interactions, including initiation and maintenance of romantic relationships (for a review, see Mahmut & Croy, 2019). Body odor seems essential when searching for a mate because, like physical appearance, it can contribute to perceiving someone as a more desirable partner. Moreover, previous studies have shown that women report body odor as an important criterion for choosing a mate and olfactory information seems more important to women than to men (Herz & Cahill, 1997; Havlíček et al., 2008). On the other hand, other recent studies have reported that men and women do not differ in the value they attach to olfaction; both sexes value the olfactory aspects of a potential mate highly (White & Cunningham, 2017; VanHatten et al., 2019). Nevertheless, evidence to the effect that women attach higher importance to olfaction than to other personal traits seems consistent (Herz & Inzlicht, 2002; Havlíček et al., 2008; White & Cunningham, 2017).

Odor cues may also have an impact on women's sexuality. Women report body odor as being sexually arousing during sexual activity more than men, who consider visual cues to be more important (Herz & Cahill, 1997; Havlíček et al., 2008). Olfactory sensitivity is positively associated with pleasant sexual experiences and, in women, also with a higher frequency of orgasm (Bendas et al., 2018). In fact, it has been documented that patients with olfactory disorders report decreased sexual desire after the disruption of their sense of smell (Schäfer et al., 2019). In ageing individuals, deteriorating olfactory functions are associated with decreased sexual motivation and less emotional satisfaction with sex but not with decreased frequency of sexual activities or physical pleasure (Siegel et al., 2021). Another study found that self-reported olfactory functions are positively associated with sexual well-being, and this relationship is mediated by pathogen disgust sensitivity (Blomkvist et al., 2021).

Experimental studies have shown that exposure to axillary odor samples from sexually aroused men activate brain areas related to the processing of sexual stimuli in women, while the same body odors were rated as less pleasant and more intense than body odors collected in a neutral condition (Zhou & Chen, 2008). In another study, women rated their genital arousal in response to sexually explicit videos while simultaneously exposed either to male body odor or no odor. They exhibited higher self-reported arousal in the no odor condition and rated it as more pleasant than the presented body odor (Alves-Oliveira et al., 2017), but it should be noted that the body odor samples in both of the abovementioned studies were collected from unfamiliar men. Exposure to the body odor of a romantic partner may lead to different results. For example, the smell of body fluids, such as semen, is perceived as strongly disgusting in a nonsexual context but can be perceived as attractive and arousing during sexual activity with a partner (Rozin et al., 1995). It seems, therefore, that in women sexual satisfaction and pleasant sexual experience may significantly influence their perception of the partner's odor.

Importantly, surveys investigating the effect of social olfaction on various aspects of relationship functioning tend to assess the global perception of the partner's odor, while majority of experimental studies focus narrowly on the axillary odor, because it is considered the most important part of the body for chemical communication in human mate choice (Lenochova & Havlíček, 2008). But the rest of the body is covered with abundant apocrine, eccrine, and sebaceous glands, which likewise significantly contribute to the perception of the partner's odor (Kippenberger et al., 2012). Moreover, people often use various fragrances: these form individually specific body odor–fragrance blends, which are in turn also involved in the olfactory perception of the romantic partner (Allen et al., 2019). To the best of our knowledge, there is currently no study investigating whether and how the partner's various odors affect intimate and sexual activities within heterosexual romantic relationships. To

explore this area in depth, we conducted semi-structured interviews to qualitatively map the range of the phenomenon (Creswel, 2009) in a way that would have the potential to generate unanticipated insights (Braun & Clarke, 2006).

The main aim of this study was to explore whether and which odor types of the partner are perceived by women within either intimate or sexual contexts. Further, we wanted to investigate whether and how these odors affect women's perceptions, including emotions, and to what extent they are specific to particular contexts. Importantly, this study considers both the perspective of currently single women and women in a committed long-term relationship to capture a wider range of data.

METHODS

Participants

A total of 40 participants took part in our study: 20 women in a committed long-term relationship and 20 currently single women. Criteria for participation were Czech nationality, age 18–40 years, not having any olfactory dysfunctions, heterosexually self-identified, being in a committed long-term relationship (minimal relationship length of six months, perceived by participants as having a perspective for the future) or being single (and less than 18 months after the end of the most recent committed relationship to prevent recall bias; Liang & Horn, 2020).

Women in a relationship were either dating (N = 13) or married (N = 7). Six out of the women in a committed relationship had children. Participants' age, education level, employment status, and length of the relationship, cohabitation, and relationship, as well as sexual satisfaction are reported in Table 1.

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Insert Table 1 about here.

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The sample size of 40 participants was established before the recruitment process. No further data collection was necessary because analysis of the data indicated a theoretical saturation of the themes (Braun & Clarke, 2021).

Interviews

Each semi-structured interview consisted of four parts. The first, second, and fourth part targeted both groups of participants, i.e., both the single women and those in a relationship. The third part of the interview targeted only single participants, focusing on their perception of their partner's odor during the period of relationship breakup. The first part focused on the women's perception of their partners' odor during the formation of the relationship, the second asked about women's perception of their partners' odor during of the relationship, and the final part investigated the participants' general level of odor sensitivity. (For the full list of questions, see Supplementary material.)

In June 2021, we conducted a pilot study with three single women aged 22–26 years (M = 24.33 years, SD = 2.08) and one woman (25 years old) engaged in a long-term relationship. Our aim was to assess whether participants would find the interview questions clear and comprehensible. Based on the responses father during this pilot stage, we have elaborated some of the interview questions. All interviews, including pilot ones, were conducted and recorded by the first author, who is experienced in interview techniques ([blinded for review]).

Procedure

Participants were recruited online via social media (Facebook research groups, Instagram), leaflets distributed at the [blinded for review], and by snowball sampling. Respondents completed an online contact form (built on a Qualtrics platform) with information about the study, followed by an informed consent form and a sociodemographic questionnaire. Maximum variation sampling (Suri, 2011) was employed regarding participants' age, education level, relationship status, relationship length, length of cohabitation, and the number of children (see the Participants section).

Semi-structured interviews were conducted in June–October 2021. Of the 40 interviews, 14 were held in-person and 26 online, depending on participants' preference associated with the ongoing Covid-19 pandemic. All participants first expressed their willingness to be interviewed by signing an online informed consent form. Interviews ranged in length from 30 to 81 minutes (M = 50.12 minutes; SD = 12.88). Interview length varied depending on the exhaustivity of participants' responses, which required less further probing and/or follow-up questions such as are envisaged in the method of semi-structured interviews (Kallio et al., 2016).

Participants were compensated for their time by 200 CZK (app. 10 USD). All procedures were conducted in accordance with the Helsinki Declaration. The study was approved by the [blinded for review] (approval number 21/10).

Data analysis

The interviews were transcribed by individual team members using the Happy Scribe transcription software (Bastié & Assens, 2017). Subsequently, the first author checked all the transcripts and transferred them to the ATLAS.ti qualitative data analysis software (Version 22.2.5.0). Examination of the data was guided by a thematic analysis driven by the researcher's theoretical or analytic interest (Braun & Clarke, 2006). This approach is based

on an intersection between the data and contextual and theoretical interpretation of the researcher (Braun & Clarke, 2021). In line with the semantic (descriptive) approach, particular themes were generated from the data and strongly linked to codes (Braun & Clarke, 2006). As recommended by Nowell and colleagues (2017), thematic coding proceeded in phases (Braun & Clarke, 2006) in order to establish trustworthiness.

In the first phase, we familiarized ourselves with the data by listening to the recordings and by repeated independent reading of interview transcripts by the two coders ([blinded for review]). In the second phase, the two coders jointly generated initial codes from the first four transcripts. This was followed by a systematic discussion and code comparison aimed at giving full and equal attention to each data item in the dataset (Braun & Clarke, 2006). Then each coder separately coded two interviews (with one single woman and one woman in a long-term relationship) to calculate inter-code agreement, which was $\alpha = 0.67$ (recommended level of Krippendorff's alpha is $\alpha = 0.667$; Krippendorff, 2004). The coders then equally divided the further transcripts to be coded (12 transcripts per coder) to establish the codebook. After reaching consensus on the codebook, the coders split the remaining eight transcripts and coded them while continuousy communicating about the process. A second inter-coder agreement was calculated with respect to another interview after each coder had completed coding at least 10 interviews (out of a total of 40) to guarantee reliable data coding. The second calculated Krippendorff's alpha value was $\alpha = 0.71$. Finally, the first author checked and revised the codebook, that is, merged some codes with overlapping content and duplicated names, and renamed some codes for better understanding.

The coding process was accompanied by the method of memo writing (researcher's diary notes), that is, by noting thoughts which came up while getting through the interview

content and relevant literature. These were used in the subsequent phases (Kalpokaite & Radivojevic, 2019), when the two coders discussed further modifications of the codebook.

At the beginning of the coding process, we have developed a conceptual framework aimed at capturing what was to be coded regarding the research questions (Kalpokaite & Radivojevic, 2019). The following aspects were coded within both the intimate (proximity of the partners with no sexual contact) and (specifically) sexual contexts: odor type (e.g., sweat), odor location (the part of partner's body or some external source, such as deodorant), and women's perception of the odor type and situation (e.g., oral sex). By putting a prefix on the code name, we distinguished between the stages of the relationship: relationship formation, ongoing relationship, the breakup period, and other, that is, occasions when respondents spontaneously reported about their ex-partners or shared experiences about the odor of a new potential partner after the breakup of a previous relationship (in the case of single women). For analyses in this study, we only used information pertaining to the stage of relationship formation and ongoing relationship, whereby the two stages were – in accordance with objectives of the study – merged. Due to the scope and aims of the current paper, we omitted the coding of partner's body parts or external sources from the analysis.

To underscore the relationships between the codes for subsequent analysis, we built "hyperlinks", which linked the odor type to odor location, women's perception, and situational context. Subsequently, we linked the situation to the women's perception. In the final phase of the analysis, we selected key odor types and situations not only based on their frequencies but especially regarding their number of links across the codes. Further, we used the network function in the ATLAS.ti to display which selected odor types are perceived in specific intimate or sexual encounters and projected the codes of women's perceptions to see how a particular odor is perceived within a specific intimate or sexual encounter. In the subsequent step, we grouped perceptions with higher frequencies and links and formed seven

categories based on similarity of their content. Interestingly, we found that the partners' odors affect not only women's emotional state but also their behavioral response, that is, we found that women react to odor exposure in various ways.

Some participants found it difficult to distinguish between the odor of shampoo and shower gel and between deodorant and cologne. In view of the general similarity between these two types of cosmetics, we have therefore merged the shampoo with the shower gel and deodorant with the cologne. Similarly, we merged the smell of hair and musk into natural body odor.

RESULTS

Key odor types perceived within specific intimate and sexual contexts

Our analysis revealed four key types of natural odor: body odor, sweat (due to heat, increased physical activity, or psychological stress), genital odor, and semen odor. Further, we identified three key types of fragrances: cologne, shower gel, and laundry agents (detergent, washing gel). Both natural odors and fragrances were perceived by women in various intimate and sexual contexts. We distinguished between the following types of intimate contexts: hugging, kissing, cuddling, and (passively) lying side by side. For sexual encounters, we differentiated between sexual intercourse, oral sex, and ejaculation (for a detailed description of the types of odors perceived within specific contexts, see Fig. 1).

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Insert Figure 1 about here.

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Odor's perception

We found that women's perception of their partner's natural odor was context-dependent. We classified odor experience as evoking a) pleasant feelings; b) unpleasant feelings/disgust; c) feelings of security and home; d) as being attractive/arousing; e) accepted as being part of the partner or activity; f) neutral or not consciously perceived; evoking either g) approach/positive behavioral response (woman wants to be closer to the partner) or h) avoidance/negative behavioral response (woman stops the intimate/sexual activity).

Partner's odors evoking pleasant feelings

Both partner's natural odor and fragrances were perceived by some women as pleasant depending on their occurrence in intimate or sexual encounters, as evidenced by the following testimony:

> That means like natural body odor, like sweat [perceived within a sexual context]. It is actually quite funny when I remind myself how unpleasant some armpit odor can be (…). But actually, I find my partner's sweat quite pleasant, sometimes I sniff his armpits for fun and I just don't mind, I don't find it unpleasant. (F13, woman in a relationship, 23 years)

Partner's odors evoking unpleasant feelings/disgust

Several women, even those who reported pleasantness of odors, mentioned that occasionally they find all odor types (except for shower gel and laundry agents) unpleasant, even disgusting, within any intimate or sexual context. In particular, this pertained to body odor, sweat, semen odor, and to "smelling stale" for the genital odor and cologne. A respondent (F1, 24 years) involved in a relationship reported why she perceives her partner's genital odor as disgusting:

> Well, I find it [the odor of partner's genitals] a little bit disgusting. As if, sort of like fuggy and stale or (chuckles), like, I don't know… A person sweats, right, so he's just like sweaty down there [in the genital area], and then there's, well, loads of bacteria when you think about it. Well, yes, it stinks because it simply produces various metabolites, that's my association,

> that I just don't wanna put in my mouth something that just stinks and that could be musty and not very clean.

To illustrate the perception of cologne, a single woman (F40, 23 years) commented on her ex-partner's cologne: "In the beginning – later he used that one [cologne] occasionally – but he had more colognes, and once when he used a particular one, it just smelled like a musty handbag from your granny's closet (laughs). It was just a type of cologne."

Interestingly, the specific odors of sweat after workday or sport were reported as most unpleasant/disgusting and were not tolerated (if mentioned) by the women in our sample with the exception of two. One respondent claimed:

> I don't like his [the partner's] smell when he communicates with me after some intensive exercise, like if he wants some intimate touching, for example, after exercise, when he's sweaty or after physical work, then I don't like his smell (…). (F11, woman in a relationship, 20 years)

Partner's odors evoking feelings of security and home

Body odor, sweat, genital odor, and cologne all evoked feelings of security for some of our female respondents. Body odor, sweat, cologne, and laundry agents were reported as evoking feelings of home and all types of odors except for semen odor and genital odor, i.e., body odor, sweat, cologne, shower gel, and laundry agents were also reported to have a calming effect on some of the women in our sample. A single woman (F39, 39 years) reported perceiving ex-partners' cologne as a feeling of home and security:

> I've already mentioned it somewhere that for me, it's [ex-partner's cologne] a feeling of home and security and kind of... something else for sure... Well, to be honest, I had the impression many times, especially at the beginning of the relationship, that the way he smells to me, that maybe I'm more in love with his scent than with him. That I was addicted to that scent so often that I wasn't so much looking forward to seeing him as to smelling it [ex-partner's cologne] again.

Attractive/arousing partner's odors

Most types of odors were reported by some women as arousing (except for the semen odor) and attractive (except for the odor of semen and shower gel) in all intimate encounters with the exception of passively lying next to the partner and in all sexual contexts, except for ejaculation. One female participant in a relationship (F20, 36 years) reported: "Well, it [partner's body odor and sweat] was clearly stimulating to some further activities because the smell was just so raw. It was definitely an arousing scent that was related to sexuality."

Acceptance of partner's odors as being a part of the partner or activity

A proportion of single women and women in a relationship reported that they accept the odor of sweat, genitals, and semen as part of all sexual encounters.

> I avoided them [the odor of ex-partner's genitals and semen] less and less, or I guess I never avoided them, but somehow more and more… I found it completely natural, something that simply belongs to the... hm, the sexual activities, actually. It's a part of it I don't mind there and perhaps, actually... er, well. I just didn't mind it (laughs). (F27, single woman, 22 years)

Similarly, another woman reported:

> I guess, I don't need to evaluate it [partner's odor of genitals and semen] somehow. I just accept it as completely normal. Just like I wouldn't judge how much saliva he'll have or how he'll taste during kissing, I don't judge this either. I certainly don't find it disgusting, but then again, I don't think you'd smear yourself with it either. I just take it as a natural part of lovemaking. I don't mind it, it doesn't bother me, but I probably wouldn't want it as a perfume. (F20, in a relationship, 36 years)

Moreover, body odor, sweat, and shower gel were reported to underline the sexual or intimate contexts. A single woman (F38, 24 years) reported: "Well, as the partner's natural body odor was a big factor in why the hugging was so pleasant."

Two odors were associated for some women with satisfying the partner during: the partner's body odor in the context of kissing, cuddling, or sexual intercourse, and the odor of semen (solely) in the context of sexual intercourse.

> I just kind of took it [the odor of partner's semen] as part of it, part of the partner, and like … I just find it basically strange that I should in any way perceive it disgusting. But... (she thinks) I guess I don't know whether it's really about the smell as such. Maybe it's about the good feeling, right? Then, in retrospect, I kind of associated that scent with situations where the partner was satisfied. So it actually satisfied me in a way, didn't it? That's probably how I would say it. (F3, woman in a relationship, 20 years)

Finally, some respondents found that the body odor and cologne somewhat reminded them of the presence of the partner during cuddling.

Neutral or no perception of partner's odors

All types of odors were also mentioned as being perceived as neutral. For instance, a woman in a relationship (F11, 20 years) remarked: "… the deodorant was probably more neutral for me because most of these deodorants are really quickly recognizable since we simply smell them everywhere, so somehow I didn't even subconsciously rate him based on that."

Alternatively, partner's odors were not reported as clearly pleasant or unpleasant by some of our female respondents. For example, a single woman (F8, 20 years) reported: " (…) it's [the odor of partner's genitals] probably neutral, because it's not like it's pleasant, it's not like I really hate it, it's not that either. It's like I perceived the smell but probably without any emotions..."

Although most women were able to report specific odor types associated with intimate or sexual contexts, some women did not perceive any of the natural body odors or fragrances (except for laundry agents, which were not reported as not perceived). They either did not recall them or did not think that particular body part has any odor: "No, no, no, I don't think it [partner's genitals and semen] has any scent at all" (F17, woman in a relationship, 24 years).

Alternatively, the women simply did not perceive any odor. One of our female respondents described a combination of some of the possible reasons:

> I don't notice the sweat, and I probably don't notice the body odor either. Now I'm wondering if it's because my husband doesn't have a strong smell or because my sense of smell is so numb. I can't recall. (F18, in a relationship, 33 years)

Partner's odors affecting women's behavioral response

In some women in our sample, the perception of body odor or cologne evoked a positive behavioral response, that is, they wanted to be closer to their partners during hugging, while others reported that body odor or the odor shower gel improved their experience during sexual intercourse. Some female respondents tended to seek out body odor, the odor of sweat, genitals, or cologne, or shower gel during hugging, cuddling, lying side by side, or sexual intercourse. For example, one woman spoke about actively seeking her partner's genital odor:

> And… yes, I often intentionally sniffed him between his legs (laughs)... Well, it was, I found it [the odor] so intoxicating, simply pleasant that … and it was the first time I did [oral sex] to a guy. Before, it simply never occurred to me it could be stimulating, but now it was really tempting to do so... So, I just simply sought the scent he has down there. (F31, single, 30 years)

In contrast, in some women in our sample the perception of their partners' body odor caused avoidant/negative behavioral response, whereby they lowered the number of intimate or sexual encounters. The perception of body odor, sweat, or genital odor caused some women to stop a particular activity, either an intimate one (kissing, hugging, cuddling) or sexual intercourse. Some female participants reported that the presence of body odor and semen odor caused them to have to force themselves into sexual intercourse. Others refused to hug or have sex with their partner because of the partner's body odor, sweat, genital odor, or semen odor. Some women stated that their perception of partner's body odor or genital odor was such they would kiss him or perform oral sex only if the partner had previously washed

himself. Some women from our sample even reported that would outright send their partner to take a shower before allowing intimacy or sexual intercourse (except for kissing) to continue or even start, because they smelled the partner's sweat, genital odor, or semen odor.

> (…) but I'd always tell him [the partner] "please, could you at least take a shower." I have quite a problem with that, but without it I just can't at all... Like, I absolutely loathe the partner at that moment when I smell the sweat from him, because usually when he comes home from work – he has a pretty hard job – he stinks a lot. Then there's still the smell of that never-ending, never-ending deodorant, but then of course when I want to do it with him... Oh well, if it's some stroking, kissing, I don't really care, but then when it comes to sex as such and so on, then that's it... (F23, woman in a relationship, 31 years)

Modulators of odor perception

In some cases, the negative perception of partner's odor is due to some modulators. Overall, women's perception of both the partner's natural odors and cologne was negatively influenced by too high/low intensity, the smell of cigarette smoke, alcohol or aromatic foods, insufficient or excessive hygiene, or partner's illness. For example, some women reported that they refuse intimacy or sex or, in particular, that they limit kissing during sexual intercourse when they smell alcohol or aromatic food from their partner:

> (…) I found it unpleasant when he one night came drunk and wanted to touch me sort of more. So, it was unpleasant because he just reeked of the booze. But that probably cannot be clearly linked to the natural body odor or the cologne or something like that. (…) I think I brushed him off quite strongly and said, "Ugh, please, just go away with these notions." Or, I don't know if I actually sent him to brush his teeth or let him turn to the other side. (F13, woman in a relationship, 23 years)
>
> (…) now that you mentioned the aromatic food, I actually realized that then when he [the partner] eats some spicy food and the like, then sometimes I find the smell of his semen unpleasant (…) but like I was saying, after such meals, he'll have to invent some other

> ending [instead of oral sex with ejaculation in the woman's mouth]. (F4, woman in a relationship, 24 years)

Most of our female participants did not spontaneously report that using hormonal contraception influences their perception of partner's odor. Nevertheless, some women noticed a negative effect of hormonal contraception on their perception of the partner's odor:

> It seems to me that the smell [partner's body odor and cologne] was more intense then, that the hormonal contraception kind of reduces those smells. … It takes away a lot of things in the sense that one isn't so receptive to, well, smells and some sensations … I'd say one's just not so perceptive. But that also depends on the type of contraception, right, there are millions of types today, right. (F23, woman in a relationship, 31 years)

DISCUSSION

Based on semi-structured interviews with 20 single women and 20 women in committed relationships, we found that women consider both partners' natural odor and artificial (fragrance) odor important within both intimate and sexual contexts. Specifically, this study identified four main types of natural odor of male partners, namely body odor, sweat, genital odor, semen odor, and three key types of artificial (fragrance) odors, namely cologne, shower gel, and laundry agents. We have investigated the perception of these odors by female partners, our respondents, in both intimate (hugging, kissing, cuddling, lying side by side) and sexual (sexual intercourse, oral sex, ejaculation) contexts. The perception of individual odors was affected by the situational context (the type of encounter and proximity it involves) and by the odor intensity, evoking various emotions (e.g., pleasant feelings, disgust) and behavioral responses (e.g., avoidance of the odor) in the women. In some cases, women's perception was negatively modified by the odor's high/low intensity, the smell of cigarette smoke, consumption of alcohol or aromatic food, insufficient or excessive hygiene, or the partner's illness. Our findings could thus be used in future studies employing quantitative

surveys or experimental design exploring women's perception of various partner's/male odors in various intimate situations.

Female participants in our study reported perception of both natural odors and fragrances in intimate and sexual contexts. Natural body odor is often taken to be exemplified by armpit odor or, to a lesser extent, by genital odor. Due to bipedalism, the armpit is considered an essential body site for chemical communication in humans (Lenochova & Havlíček, 2008). One also finds here the highest density of the apocrine glands, whose products significantly contribute to the formation of body odor (Groscurth, 2002), which is also why most experimental studies use armpit odor. Our results, however, suggest that such approach might be overly simplistic. We found that many women smell and sometimes also sniff various areas of their partners' bodies. Various parts of the body contain not only apocrine but also eccrine and sebaceous glands in varying in site-typical densities and products of all three types of glands seem to contribute to the overall body odor (Kippenberger et al., 2012).

Many individuals use various fragranced products and their odor also becomes part of one's personal odor. There are two main ways in which fragrances interact with the natural body odor: they can either mask the body odor or create an idiosyncratic blend. Empirical studies support the blending hypothesis show that people tend to choose perfumes fitting their body odor (Lenochová et al., 2012; Allen et al., 2016). Our results align with previous findings in that some participants reported they want their partner to use fragrances to make their natural odors more pleasant. Furthermore, participants often mentioned that partner's specific cologne, shower gel, or shampoo significantly contributes to the overall perception of partner's odor.

Most women in our sample recalled various types of their partner's odors perceived in specific contexts. Odors can easily modulate mood and one's emotions (for review see,

Delplanque et al., 2017), whereas body odors are not an exception. For example, smell of fear (body odor collected under fearful condition) can easily elicit negative emotions in the receiver (de Groot et al., 2012). Moreover, some odors were reported to affect women's emotional states such as evoking feelings of home and security. These results are supported by findings from previous studies, which had, for instance, shown that sniffing the odor of one's romantic partner reduces stress and contributes to higher sleep efficiency (Hofer et al., 2018; Hofer and Chen, 2020). The "comfort smelling" of a partner's belongings in their absence also seems to be a crucial activity for a sense of security (McBurney et al., 2006).

We further found that some partner's odors can even provoke women's behavioral response. Some of these responses take an affiliative form, where the woman wants to be closer to her partner, others a negative one, namely avoidance. Some women, for instance, openly commented on partner's odor and told them to take a shower before having sex or intimate encounter with them. Our findings are in line with prior studies on the importance of male odor perception in the functioning of relationships (Herz & Cahill, 1997). Our study has contributed to current knowledge new insights about the strength of impact which partner's odor can have on women. Partner's odor perceived as unpleasant may decrease the frequency of sexual activities, which may in turn lead to conflicts (Doss et al., 2004; Reese-Weber et al., 2015) and low relationship satisfaction (Meyer & Sledge, 2022).

In our study, we have shown that body odor perception is highly context dependent. For example, certain mostly negatively perceived odors, such as the odor of semen or genitals, were often accepted as a part of the sexual encounter. It is possible that such context-dependent change in perceived valence is due to sexual arousal, which suppresses negative feelings, including disgust (Bancroft et al., 2009). In contrast, some women in our sample reported a negative perception of specific types of their partner's sweat, for instance, after workday, workout, or when the partner is ill. We suggest that in such cases, the

sequence of cause and effect may work the other way around. Specifically, negatively perceived odors in intimate contexts, such as hugging, may suppress sexual arousal. Further, women's aversion in the case of partner who is ill may be due to activation of the behavioral immune system, whose purpose is to prevent contagion by pathogens (Schaller & Park, 2011).

Importantly, we found considerable interindividual variability in odor perception. The same type of odor would be perceived differently or even with opposite valence by different female respondents. For example, partner's body odor was perceived as pleasant by some women and as unpleasant or even disgusting by others. Some respondents found the odor of genitals disgusting, leading to refusal of oral sex, while others accepted this odor as a part of the partner or activity, and yet others found the odor arousing. It seems that the perception of various odors depends on personal, idiosyncratic experience. Similar interindividual variation has been recently reported for the perception of disgusting odors (Juran et al., 2023).

In line with prior findings (Mahmut & Croy, 2019), we found that odor perception was not linked solely to the odor itself. It was more complex: some women reported that their olfactory perception of their partners' odors depends on the particular intimate or sexual context. In some cases, respondents were unable to make a clear distinction between, for example, whether they like the odor as such or rather the situational context in which it becomes prominent.

Limitations and Future Research Directions

This study focuses on women's perception of partner's odours in heterosexual long-term relationships. Whether their experiences would be different in more casual sexual encounters or in the same-sex relationships remains to be addressed. Previous research found that male appreciation of odours during sexual activities is relatively less significant as compared to the

visual stimulation (Herz & Inzlicht, 2002; Havlíček et al., 2008). However, this does not mean that partner's odours do not influence male experiences of intimate and sexual activities and similar investigation should focus on male's perspective. It should be also noted, that due to the qualitative design of this study, no population inferences can be made (Seidman, 2006). Future quantitative studies should thus map how specific types of partner's odour affect perception in various contexts which might further influence the couple's relationship functioning (Doss et al., 2004; Reese-Weber et al., 2015).

CONCLUSIONS

To conclude, we found that both natural and artificial (fragrance) odor of the partner can importantly affect women's emotional states (pleasant or unpleasant feelings, disgust) and behavioral responses (ranging from approach to avoidance of a partner). Women's odor perception seems context-dependent, because for instance some mostly negatively perceived odors, such as semen odor or genital odor, were often accepted as part of the sexual encounter. Moreover, specific types of partner's sweat, such as after workday, workout, or when the partner is ill, were negatively perceived within intimate encounters, perhaps ultimately to avoid contagion by pathogens (Schaller & Park, 2011). Finally, the same type of odor could be perceived by different respondents in different ways or even with the opposite valence. For example, some respondents found the odor of genitals disgusting, leading to rejection of oral sex, while others accepted it as part of the partner or activity. This study thus highlights the interindividual variability and complexity in women's experience with the partner's various odors.

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Table 1 Sample demographics

		Women in a relationship	Single women
Age (years)	Range	20–39	19–39
	M	26	23.75
	SD	6.04	4.58
Education level (N)	Secondary education	8	13
	University	12	7
Current employment (N)	Student	5	5
	Part-time job	6	8
	Employed	4	6
	Unemployed	0	1
	Maternal leave	5	0
Relationship length (months)	Range	7–180	5–96
	M	61.1	24.35
	SD	50.26	20.96
Relationship satisfaction (7 = extremely satisfied)	Range	3–7	3–7
	M	5.55	4.5
	SD	1	1.4
Sexual satisfaction (7 = extremely satisfied)	Range	3–7	2–7
	M	5.4	4.85
	SD	1.27	1.69

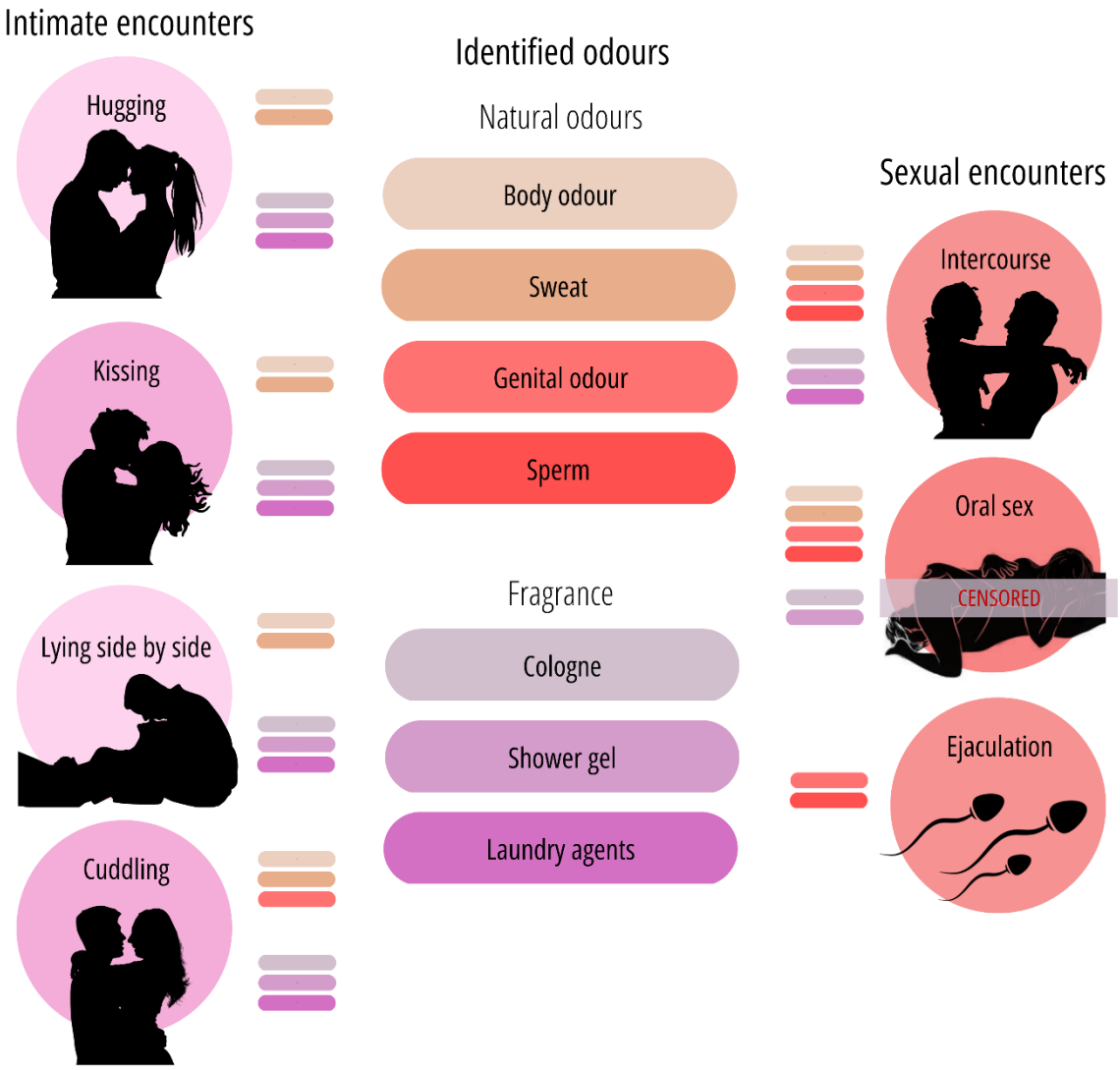

Fig. 1. Identified odors (middle column) within the intimate (left) and sexual (right) contexts. Each color bar next to the specific type of encounter represents particular type of natural odor or fragrance.

Statement of contribution

This is to confirm that Mgr. Dagmar Schwambergová significantly contributed to the following publication:

Fišerová, A., Pátková, Ž., Schwambergová., D., Jelínková, L., & Havlíček, J. (under review). The context matters: Women's experiences of their partner's odor in intimate and sexual encounters. *Archives of Sexual Behavior.*

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She contributed to the conceptualisation, manuscript writing and subsequent editing and revisions.

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

_____

doc. Mgr. Jan Havlíček, Ph.D.
supervisor

Chapter 14

OLFACTORY SELF-INSPECTION: OWN BODY ODOUR PROVIDES CUES TO ONE'S HEALTH AND HYGIENE STATUS

Evolution and Human Behavior

Olfactory self-inspection: Own body odour provides cues to one's health and hygiene status

--Manuscript Draft--

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Corresponding Author:	Dagmar Schwambergová, MSc Prague, Czech Republic CZECH REPUBLIC
First Author:	Dagmar Schwambergová, MSc
Order of Authors:	Dagmar Schwambergová, MSc
	Jitka Třebická Fialová, Ph.D.
	Jan Havlíček, Ph.D.
Abstract:	Olfactory self-inspection has been observed in various mammals, including humans. This behaviour can help individuals monitor own state, including health and hygiene. This study's aim was to explore the frequency of sniffing particular body parts and investigate possible gender differences. Further, we tested a possible evolutionary function of this behaviour, namely monitoring of health and hygiene, by investigating associations between the frequency of self-sniffing and health, hygiene, and disgust sensitivity. Respondents completed an online survey on self-sniffing behaviour, health status, hygiene habits (HI-23), and disgust sensitivity (TDDS and BODS). Self-sniffing behaviour was investigated using a purpose-built inventory which explored the incidence and frequency of sniffing different parts of own body using a verbally anchored 7-point scale. Principal Component Analysis identified three main axes of self-sniffing behaviour: social acceptability self-inspection, intimate self-inspection, and cosmetic self-inspection. Our results further show that respondents with less hygienic habits engage significantly more in intimate self-inspection (sniffing body parts such as genitals, anus, or navel). Interestingly, individuals who reported more frequent health issues sniff more frequently areas such as the armpits, feet, or own breath (social acceptability self-inspection), probably to check for possible changes in smell due to illness. Our results indicate that olfactory self-inspection probably has several evolutionary functions depending on the location from which the smell originates.
Suggested Reviewers:	Katrin Lübke Heinrich Heine University Düsseldorf katrin.luebke@hhu.de Expert on chemical communication in humans and olfactory performance.
	Ofer Perl Hebrew University of Jerusalem oferikoo@gmail.com Expert on human olfaction and memory.
	Tamsin Saxton Northumbria University TAMSIN.SAXTON@NORTHUMBRIA.AC.UK Expert on the significance of biological traits in interpersonal perceptions, and the role of chemosignals in interpersonal interactions.
	Camille Ferdenzi French national centre for scientific research camille.ferdenzi-lemaitre@cnrs.fr Expert on odor perception in humans (the variability of olfactory perception and the role of body odors in social interactions).
Opposed Reviewers:	

Title Page (with Author Details)

Olfactory self-inspection: Own body odour provides cues to one's health and hygiene status

Dagmar Schwambergová[1], Jitka Třebická Fialová[1], and Jan Havlíček[1]

[1] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic

* Corresponding author:

Tel.: +420 221951853, fax: +420 221951125

E-mail: schwambd@natur.cuni.cz

Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic

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ABSTRACT

Olfactory self-inspection has been observed in various mammals, including humans. This behaviour can help individuals monitor own state, including health and hygiene. This study's aim was to explore the frequency of sniffing particular body parts and investigate possible gender differences. Further, we tested a possible evolutionary function of this behaviour, namely monitoring of health and hygiene, by investigating associations between the frequency of self-sniffing and health, hygiene, and disgust sensitivity. Respondents completed an online survey on self-sniffing behaviour, health status, hygiene habits (HI-23), and disgust sensitivity (TDDS and BODS). Self-sniffing behaviour was investigated using a purpose-built inventory which explored the incidence and frequency of sniffing different parts of own body using a verbally anchored 7-point scale. Principal Component Analysis identified three main axes of self-sniffing behaviour: social acceptability self-inspection, intimate self-inspection, and cosmetic self-inspection. Our results further show that respondents with less hygienic habits engage significantly more in intimate self-inspection (sniffing body parts such as genitals, anus, or navel). Interestingly, individuals who reported more frequent health issues sniff more frequently areas such as the armpits, feet, or own breath (social acceptability self-inspection), probably to check for possible changes in smell due to illness. Our results indicate that olfactory self-inspection probably has several evolutionary functions depending on the location from which the smell originates.

Keywords: olfaction, self-monitoring, health status, hygiene, disgust sensitivity

1. INTRODUCTION

In humans, self-inspection (or self-monitoring) occurs in almost every aspect of social life, from public appearances and expressive control all the way to interpersonal relationships (Fuglestad & Snyder, 2009). To accurately interpret social interactions, one needs to be aware of one's own state and behaviour. Self-inspection can thus be seen a strategy that enables individuals to exert control over own current state and over how they may appear to their conspecifics (Snyder, 1979). Still, self-inspection also occurs in non-social contexts, where it is associated for instance by desire to monitor own health status (Todd & Mullan, 2014). Self-inspection may take place visually, acoustically, haptically, and olfactorily, and while olfactory self-inspection is only rarely studied in humans, its purpose may be to control various aspects of own body odour.

Various species of mammals engage in olfactory self-inspection at relatively high frequencies. It may take the form of direct sniffing of own body or indirectly by sniffing own scent marks. For example, grey wolves (*Canis lupus*) seem to recognise and discriminate their own urine marks from the marks of other familiar or unfamiliar individuals (Cazzolla Gatti et al., 2020). In rodents, who heavily rely on olfactory communication, self-sniffing during self-grooming may help them control how much information they provide to potential rivals and mates. For example, male meadow voles (*Microtus pennsylvanicus*) self-groom quite extensively in the presence of the odour of female conspecifics (Scauzillo & Ferkin, 2020). Moreover, self-grooming may boost the transmission of social information provided by the groomer (Ferkin 2019). It has been demonstrated that female lemurs (*Lemur catta*) increase the frequency of sniffing their genitals after mating. Importantly, such behaviour has also been observed outside the mating period and may therefore have roles other than sperm monitoring (Palagi, Telara & Tarli, 2003). In common chimpanzees (*Pan troglodytes*) of Mahale, sniffing has been observed during self-checking of their bodies, and it was more frequent in females (Matsumoto-Oda et al., 2007).

Another way of olfactory self-inspection was observed in primates. Great apes tend to examine and sniff their fingers after touching marks on their bodies when looking in the mirror: this could be an attempt to acquire information from a modality other than vision (Anderson & Gallup Jr., 2015). Moreover, general face-touching at intervals of about one minute has been observed in gorillas (*Gorilla gorilla*), chimpanzees, and orangutans (*Pongo pygmaeus abelli*) (Dimond & Harries, 1984).

Studies on the frequency of face-touching in humans show that individuals tend to touch their nose, mouth, or eyes about 10 to 23 times an hour (Nicas & Best, 2008; Elder et al., 2014; Kwok et al., 2015). In another study, face-touching was observed in participants seated in an experimental room after a greeting with or without the handshake. Before the greeting, participants were seated alone for some

time: they spent app. 22% of that time touching their hand. After shaking hands with an individual of the same gender, the participants brought their right hand closer to their face more frequently. After shaking hands with an individual of the opposite gender, they brought their left hand closer to their face, which may have served as a brief olfactory self-inspection of own physiological state (Frumin et al., 2015). More recently, it was shown that face-touching after a handshake took place more frequently in private settings. This study compared the handshaking interaction during a graduation ceremony and during a relaxed meeting with an experimenter in the lab. Importantly, participants brought their left (non-shaking) hand to the nose area more often than they did their right hand (Roberts et al., in press). Previous findings indicate that hand-sniffing may serve as a way of inspecting own body odour directly from the hands or a way of inspecting odour from the environment after touching the examined surface, but more studies on face-touching after handshaking need to be conducted to resolve various inconsistent findings.

Experimental studies help us understand the self-sniffing (i.e., olfactory self-inspection) of hands, but for ethical reasons one cannot observe touching and sniffing of other, more intimate body parts. Researchers must therefore rely on self-reports. In a pioneering study, Perl and colleagues (2020) found that above 90% of respondents reported sniffing their hands or armpits and 73.9% of men and 55.7% of women reported sniffing own hand after touching their crotch (Perl et al., 2020). To investigate olfactory inspection behaviours, Li and colleagues (2022a) developed the Body Odor Sniffing Questionnaire (BOSQ). Besides the body parts mentioned in their previous study, BOSQ also contains items on sniffing feet, hair, or personal items such as quilts and pillows. BOSQ consists of three factors: i) 'Self – private body odor' captures the sniffing of own private parts such as armpits, crotch, or feet, ii) 'Others' body odor' captures the sniffing of others, such as family members, partners, or friends, and iii) 'Self – common body odor' captures the sniffing of more 'public' body parts such as hair, hands, and fabrics. The results showed that Chinese respondents report a higher frequency of sniffing Self – common body odour compared to US respondents, who showed a higher frequency of sniffing Self – private body odour. Women also tended to sniff themselves more often than men did and body odour sniffing behaviours negatively correlated with age (Li et al., 2022a). Furthermore, persons who engage more in self-sniffing reported a stronger sexual desire (Li et al., 2022b). It seems, therefore, that self-sniffing is dependent on gender, age, and culture, although the context underlying this behaviour remains unknown.

Smelling oneself may function as a control of body odour quality and an inspection of a possible malodour. Body odour conveys various information about one's health status (Olsson et al., 2014), emotional state (de Groot, Semin, & Smeets, 2017), diet (Zuniga et al., 2017; Fialová et al., 2019), and hygiene. Olfactory self-inspection can thus provide an update on one's physiological and emotional

state, and this information can be perceived by other individuals as well (de Groot, Semin, & Smeets, 2017). Moreover, olfactory self-inspection may be the first in line when checking own hygiene, for instance further usability of a previously worn T-shirt or the need to use a fragrance to smell nice to others. Yet it might also play a crucial role in inspection of own health status because various diseases lead to metabolic changes that can be subsequently perceptible in body excretions, such as urine, faeces, breath, or sweat from different areas of the body (Havlíček et al., 2017, Shirasu & Touhara, 2011). The behavioural immune system detects potentially threatening stimuli based on various cues (Schaller & Duncan, 2016). Accurate detection is followed by a negative affective response in the form of disgust, which helps the individual avoid the risk. Although the primary role of this system is to recognise potentially hazardous stimuli, including infected conspecifics, it may also play a role in monitoring own health status. This would be beneficial because the ill individual can withdraw from social interactions and decrease the likelihood of transmitting pathogens to others, including relatives.

Our study had three main aims. First, we wanted to identify which body parts are sniffed more often than other body parts. Second, we investigated possible gender and age differences by comparing male and female responses regarding the frequency of olfactory self-inspection. Based on previous findings (Li et al., 2022a,b), we expected that female respondents would engage in self-sniffing more often, while male respondents would sniff more frequently their private areas (as reported by Perl et al., 2020). We have also expected that older participants would report a decline in the frequency of self-sniffing (as reported by Li et al., 2022a). The third – and perhaps the most important – aim of the study was to investigate possible evolutionary functions of olfactory self-inspection, in particular the monitoring of health and hygiene status. To that purpose, we tested associations between the frequency of self-sniffing, health status, hygiene, and disgust sensitivity. We predicted that respondents with a higher hygiene score will engage in more frequent olfactory self-inspection in order to effectively control the state of their hygiene using their sense of smell. We further predicted that individuals with more self-reported health issues will sniff themselves more often in order to monitor their health status (for example from their breath). Finally, we predicted that individuals with a higher overall disgust sensitivity and disgust elicited by body odour will sniff themselves less and this lowering of self-sniffing will pertain mainly to body parts such as the armpits, feet, or private areas, which tend to be more odorous.

2. METHODS AND MATERIALS

The study was conducted online, in Czech, and consisted of set of questionnaires on olfactory behaviour and related measures. The project was approved by the Institutional Review Board of

Charles University (approval no. 2020/30). Informed consent was obtained from all respondents before starting the survey.

2.1. Pilot study

To explore the body parts and contexts relevant for self-sniffing, we ran a pilot study. We included 7 items on body parts or previously worn clothes, which were used previously by Perl and colleagues (2020), and added another 11 items on body parts based on the distribution of skin glands (e.g., back or chest). The whole survey on sniffing own body contained 39 items. The first part of the survey consisted of 18 items and investigated the incidence and frequency of sniffing of different parts of own body directly (e.g., 'Do you ever sniff your armpits?') or indirectly, that is, via hand contact or previously worn clothes using a 7-point scale (1 – 'never or almost never', 7 – 'several times a day'). Then the survey contained 21 open-ended questions regarding the context of this behaviour (e.g., 'In what situations do you smell your own armpits?'). Data collection lasted from January to mid-February 2021. A total of 124 Czech respondents completed the pilot study (77 women: M_{age} = 27.1, SD = 11.07 and 47 men: M_{age} = 24.2, SD = 4.80). Before the survey on self-sniffing, respondents first completed a short sociodemographic survey (age, gender, and education).

Qualitative data from the open-ended questions obtained in the pilot study were then used to prepare an adapted version of the inventory. Due to low occurrence, we left out items on the frequency of sniffing own back (N = 0) and chest via hand contact (N = 6). For these body parts, items regarding the context of this behaviour were then also excluded from the final version of the inventory. Results of the pilot study can be found in Supplementary Materials S1. Analysis of data on the contexts of self-sniffing will be presented elsewhere.

2.2. Participants

We have recruited participants aged over 18 years via an e-mail contact list compiled from our previous studies and via social media (Facebook). Potential participants were invited to take part in a study on olfactory behaviour. A total of 314 Czech respondents started the survey, but 98 individuals did not complete it (i.e., failed to complete over one-fifth of the questionnaire) and were excluded from further analyses. Further, we excluded 7 outliers in age (older than 38 years) with values larger than 1.5 * inter-quartile range from the upper hinge in the box plot. This resulted in a final sample of 209 respondents (153 women: M_{age} = 24.0, SD = 4.82 and 59 men: M_{age} = 24.2, SD = 4.70). Participants received no reimbursement.

2.3. Procedure

The online survey was run on the Qualtrics platform and distributed from November 2021 until June 2022. First, respondents completed a short sociodemographic questionnaire followed by the self-sniffing inventory (e.g., 'Do you ever smell your own hands?'). Subsequently, we asked participants to assess their sense of smell and pleasantness of their body odour. This followed by a set of standardised measures in a random order (the Three Domain Disgust Scale, Body Odor Disgust Scale, Hygienic Behavior Inventory, The Big Five Inventory–2-S, and an inventory on long-term and current health).

2.4. Measures

2.4.1.The sociodemographic questionnaire

To obtain data on basic sociodemographic characteristics such as age, gender, education, and occupation, participants completed a sociodemographic questionnaire comprised of 7 items. For the full list of sociodemographic items, see S2 in the Supplemental Materials.

2.4.2.Self-sniffing inventory

To assess self-sniffing behaviour, we asked the participants how often they sniff their various body parts and previously worn clothes and in what the context the smelling of each body part or clothes takes place. The inventory consisted of 35 items, 16 of which covered the particular body parts using question such as 'Do you ever smell your armpits?' or 'Do you ever smell your hand after touching your genitals?' (see Table 1). Each item was rated on a verbally anchored 7-point scale. Response options were: 'never' (scored as 1), 'almost never/less than a few times a year', 'several times a month', 'once a week', 'several times a week', 'every day', 'several times a day' (scored as 7). Another 16 items covered the context in which self-sniffing of a particular body part occurs. If the respondent chose the option 'never' for a given body part, the list of contexts was skipped. The options depended on the particular body part (e.g., for hands there were 16 options while for navel only 11) but our aim was to match the contexts across the body parts as much as possible. As a result, 10 options were identical for all body parts and 8 options were identical across all worn clothing. The rest was specific, so that for instance for hands there were specific options such as 'after contact with my own body' or 'after contact with someone else's body', while for genitals there was an additional option 'full check of my own odour (health control)'. The remaining three items covered the parts of T-shirts, trousers, and hands which respondents smelled if they did not previously choose the option 'never' (see Table 2).

Do you ever smell your…
1. hands?
2. armpits?
3. hair?

4. breath?
5. feet?
6. hand after touching your armpit?
7. hand after touching your hair?
8. hand after touching your genitals?
9. hand after touching your anus?
10. finger after sticking it in your ear?
11. finger after sticking it in your navel?
12. hand after touching your neck?
13. T-shirt after you wore it?
14. trousers or skirt after you wore them?
15. socks after you wore them?
16. underwear after you wore it?

Table 1: The first part of the questionnaire covering the body parts and frequencies of self-sniffing. Each item was rated on a verbally anchored 7-point scale. Response options were: 'never' (scored as 1), 'almost never/less than a few times a year', 'few times a month', 'once a week', 'several times a week', 'every day', 'several times a day' (scored as 7).

2.4.3.Three Domain Disgust Scale

The Three Domain Disgust Scale (TDDS) (Tubyr et al., 2009) is a self-report measure with 21 items consisting of three subscales (pathogen disgust, moral disgust, and sexual disgust), with 7 items in each subscale. The items were rated on a 7-point scale ranging from 1 (not disgusting at all) to 7 (extremely disgusting). The total score could range between 21 and 147, and each subscale between 7 and 49. Higher scores indicate higher levels of disgust sensitivity in total and in individual subscales. The Czech language mutation had been translated by two independent bilingual translators (Polák, Landová & Frynta, 2018) and we had successfully used in our previous research (Schwambergová et al., 2023).

2.4.4.Body Odour Disgust Scale

The Body Odor Disgust Scale (BODS) (Liuzza et al., 2017) is a self-report questionnaire with 12 items on body odours eliciting disgust. It consists of two subscales depending on whether the odour source is external (e.g., 'You are sitting next to a stranger and notice that his feet are very smelly') or internal (e.g., 'You are alone at home and notice that your feet are very smelly'). To align the inventory with the other measures, we used a 7-points scale ranging from 1 (not disgusting at all) to 7 (extremely disgusting). The score for each domain was calculated as the mean value and could range between 1 and 7. We used the scale in the Czech language mutation in our previous research for which we

translated the inventory into Czech and verified its accuracy by backtranslation (Schwambergová et al., 2023).

2.4.5.Hygiene Inventory

To assess hygiene-related behaviour, we used the self-report Hygiene Inventory (HI-23) (Stevenson et al., 2009), which contains 23 items. The individual items load into five subscales: general hygiene (eight items), household hygiene (three items), food-related hygiene (three items), handwashing technique (five items), and personal hygiene (four items). Participants rate each item on a 4-point verbally anchored scale ranging from one to four. The response options for general hygiene and food-related hygiene subscales are 'never' (scored as 1), 'occasionally', 'usually', and 'always' (scored as 4). Only the item 'On an average day, approximately how many times do you wash your hands?' (general hygiene subscale) has the following options: 'never' (scored as 1), '1 to 5', '6 to 10', and '11+' (scored as 4). Response options for the household hygiene subscale are: 'never' (scored as 1), 'once', 'twice', and 'three or more times' (scored as for 4). For the item 'When you wash your hands, approximately how long do you wash them for?' (handwashing hygiene technique subscale) the response options are: 'under 5 seconds' (scored as 1), '6 to 10 seconds', '11 to 20 seconds', and '21 seconds or more' (scored as 4), while the item 'Do you use an antibacterial gel or wipes to clean your hands?' (handwashing hygiene technique subscale) has response options: 'never' (scored as 1), 'rarely', 'sometimes', and 'often' (scored as 4). Finally, the subscale covering personal hygiene has response options: 'never' (scored as 4), 'rarely', 'sometimes', and 'often' (scored as 1). In 6 items, we provided additional response options, such as 'not sure' in the household hygiene dimension, which was scored as an average score (i.e., 2.5). Additional response options regarding hygiene-related behaviours such as 'I never eat with my hands' or 'I never touch pets during the food preparation' were scored as the highest (i.e., 4) in the other three items. The overall score is calculated as a mean, ranging from 1 to 4. Higher scores indicate greater reported hygiene-related behaviour. We used the scale in a Czech mutation in our previous research for which we translated the inventory into Czech and checked its accuracy by backtranslation (Schwambergová et al., 2023).

2.4.6.Health-related questionnaire

The questionnaire on health contains nine items about the incidence of common health issues such as headaches, colds, or fatigue (e.g., 'How often do you suffer from headaches?' or 'Do you use any medication prescribed by your family doctor?'). Respondents rate each item on a verbally anchored 7-point scale. Response options are: 'I don't suffer from this issue at all' (scored as 0), 'less than once a year', 'once a year', 'twice a year', 'every three months', 'once a month', 'once a week', and 'more

often' (scored as 6). The overall score could range from 0 to 54, with higher scores indicating a higher incidence of health issues. For the full list of health-related items, see Schwambergová et al. (2023).

Participants also completed the Big Five Inventory–2-S to assess personality characteristics but results from that inventory and from the contextual part of the Self-sniffing inventory will be presented elsewhere.

2.5. Statistical analysis

All statistical tests were performed using Jamovi v. 2.3.13. software (The jamovi project, 2021; https://www.jamovi.org). We performed a Principal Component Analysis (PCA) with varimax rotation to reduce the dimensionality of frequency data from the Self-sniffing inventory. To assess possible gender differences in the frequency of self-sniffing, we employed a series of ANOVAs, where the final components of self-sniffing frequency (PCA1: Social acceptability self-inspection, PC2: Intimate self-inspection, and PCA3: Cosmetic self-inspection) served as dependent variables and gender as a fixed factor. To test associations between the age and all components of the self-sniffing frequency, we employed Pearson's correlations. Analogically, to assess possible effects of the health score, hygiene score, and the disgust scores [TDDS, BODS]) on self-sniffing frequencies, we employed linear regressions. To account for a possible effect of gender, we included this variable into the regression models. TDDS and BODS overall score was analysed separately and was not entered into the same regression model as its subdomains (TDD-pathogen, moral, and sexual or BODS-internal and BODS-external).

3. RESULTS

See Table 2 for the characteristics and descriptive statistics of all variables, such as participants' age, occupation, education level, family income, incidence of self-sniffing particular body parts, health score, personality traits, disgust sensitivity, and hygiene-related behavior.

Characteristics of the sample		**N**	**%**
Occupation			
	Employed	77	36.7%
	Student	153	72.9%
	Maternal leave	13	6.2%
	Pensioner	0	0%
	Unemployed	3	1.4%
Education level			
	Elementary	3	1.4%

	Secondary school	129	61.5%
	University	79	37.1%
Family income (per month)			
	under 20,000 CZK	43	20.6%
	20,000–45,000 CZK	67	32.7%
	46,000–75,000 CZK	59	28.2%
	76,000–100,000 CZK	24	11.5%
	over 100,000 CZK	16	7.7%

Parameter name	Mean	SD	Range (min, max)
Age	24.03	4.78	18, 38
Hands (F)	4.94	1.72	1, 7
Armpits (F)	4.46	1.46	1, 7
Hair (F)	3.78	1.88	1, 7
Feet (F)	2.34	1.13	1, 6
Breath (F)	3.74	1.7	1, 7
Armpit via hand (F)	3.05	1.67	1, 7
Hair via hand (F)	2.39	1.74	1, 7
Genital via hand (F)	3.74	1.74	1, 7
Anus via hand (F)	2.19	1.29	1, 7
Ear via finger (F)	2.19	1.56	1, 7
Navel via finger (F)	1.81	1.25	1, 7
Neck via hand (F)	1.66	1.16	1, 7
Worn T-shirt (F)	4.89	1.23	1, 7
Worn trousers or skirt (F)	2.71	1.46	1, 7
Worn socks (F)	3.2	1.53	1, 7
Worn underwear (F)	2.87	1.57	1, 7
Health score	26.93	8.94	2, 55
TDDS	63.11	17.9	11, 105
TDD-pathogen	21.85	7.28	2, 41
TDD-moral	25.39	9.75	0, 42
TDD-sexual	15.88	8.15	0, 39
BODS	4.64	1.25	1.42, 7
BODS – internal	3.84	1.51	1, 7
BODS – external	5.44	1.22	1.5, 7
Hygiene-related behavior	2.98	0.34	2.06, 3.77

Table 2: *Descriptive statistics for respondents' age, occupation, educational level, family income, sniffing frequencies of each body part (marked with F), personality traits, health status, disgust sensitivity (TDDS and BODS) and hygiene-related behavior.*

3.1. Self-sniffing factor structure

To explore the underlying factor structure of self-sniffing frequencies, we performed a PCA with varimax rotation. We have identified three components based on the criterion of eigenvalue greater than 1 (5.48, 1.67, and 1.34, respectively), which together explained 53% of total variance. Factor 1 was comprised of eight items (items 2, 4, 5, 6, 13, 14, 15 and 16) and accounted for 23.8% of the variance; Factor 2 was comprised of four items (items 8, 9, 10 and 11) and accounted for 16.5% of the variance; Factor 3 was comprised of four items (items 1, 3, 7 and 12) and accounted for 12.7% of the variance. Based on the content of the loading items, Factor 1 was named 'social acceptability self-inspection', Factor 2 was named 'intimate self-inspection', and Factor 3 'cosmetic self-inspection'. The actual eigenvalues from PCA are shown in Fig. 1, while component loadings are depicted in Table 3.

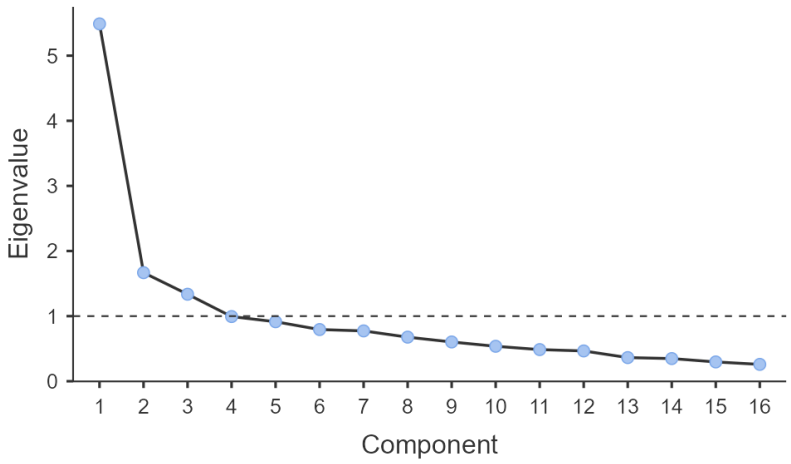

Fig. 1: *Scree plot depicting eigenvalues for individual factors.*

	Component			
	1	2	3	Uniqueness
Worn socks	0.728	0.356	-0.039	0.342
Worn T-shirt	0.711	0.082	0.181	0.455
Worn trousers or skirt	0.681	0.090	0.016	0.528
Worn underwear	0.638	0.402	-0.070	0.427
Armpits	0.665	0.040	0.275	0.480
Feet	0.623	0.031	0.269	0.538
Breath	0.640	0.084	0.268	0.510
Armpit via hand	0.554	0.263	0.323	0.519
Anus via hand	0.215	0.769	0.040	0.361

Ear via finger	0.077	0.651	0.393	0.400
Genitals via hand	0.343	0.702	0.124	0.374
Navel via finger	0.064	0.580	0.464	0.442
Hair	0.297	-0.394	0.564	0.437
Neck via hand	0.031	0.223	0.654	0.521
Hair via hand	0.197	0.111	0.627	0.555
Hands	0.214	0.263	0.478	0.656

Table 3: *Component loadings into three factors; varimax rotation was used.*

3.2. Gender differences in the frequency of self-sniffing

To investigate possible gender differences in the three self-sniffing factors, we performed a series of ANOVAs. We found no difference between women and men in the frequency of social acceptability self-inspection ($F_{1,204} = 0.689$, $p = 0.408$, $\eta^2p = 0.003$), but men reported a statistically significantly more frequent intimate self-inspection ($F_{1,204} = 63.8$, $p < 0.001$, $\eta^2p = 0.238$) and women reported more frequent cosmetic self-inspection ($F_{1,204} = 3.80$, $p = 0.053$, $\eta^2p = 0.018$), see Fig. 2. To account for this variable, we have added gender as a covariate to further analyses.

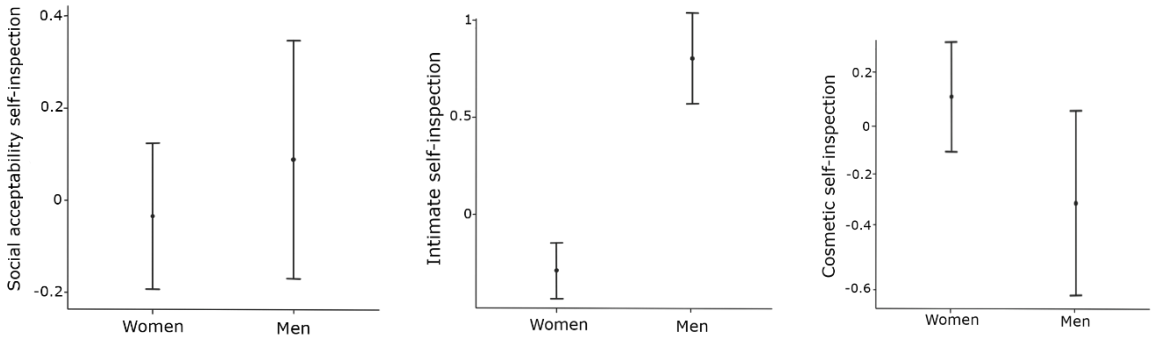

Fig. 2: *Sex differences in hygienic, intimate, and cosmetic self-inspection.*

3.3. Association between age and the frequency of self-sniffing

We found no association between age and the frequency of social acceptability self-inspection ($r = -0.122$, $p = 0.081$), intimate self-inspection ($r = 0.120$, $p = 0.085$), or cosmetic self-inspection ($r = -0.038$, $p = 0.590$). In view of these null findings, we did not add age as a covariate to further analyses.

3.4. Association between social acceptability self-inspection and the health score, hygiene habits, and disgust

A linear regression ($F(5,194) = 2.18$, $p = 0.058$, $R^2 = 0.053$) showed a statistically significant positive association between social acceptability self-inspection and the health score. In other words,

respondents who tended to sniff their worn clothes, armpits, or breath were also likely to suffer more frequently from health issues (see Table 4).

Characteristic	Predictors	F	β	95% CI (LL, UL)	t	SE	p
Social acceptability self-inspection	**Health score**	7.77	0.022	0.006, 0.605	2.789	0.008	**0.006**
	Hygienic behaviour	0.37	0.143	-0.319, 0.605	0.611	0.234	0.542
	TDDS	1.44	0.006	-0.003, 0.016	1.201	0.005	0.231
	BODS	0.36	-0.004	-0.174, 0.093	-0.601	0.067	0.548
	Gender	3.01	0.296	-0.040, 0.633	1.736	0.170	0.084

Table 4: *A linear regression testing the relationship between hygienic behaviour, health score, disgust sensitivity (TDDS and BODS) and social acceptability self-sniffing frequency controlled for gender. Df is 1 for all variables and 194 for residuals. Statistically significant associations are marked in bold.*

To see whether there is any association between the social acceptability self-inspection and the disgust domain of TDDS or BODS, we employed another linear regression ($F(6,194) = 1.18$, $p = 0.318$, $R^2 = 0.035$). It showed a statistically significant positive association between social acceptability self-inspection and moral disgust, meaning that respondents who are more willing to follow social norms sniff their worn clothes, armpits, or breath more frequently (see Table 5).

Characteristic	Predictors	F	β	95% CI (LL, UL)	t	SE	p
Social acceptability self-inspection	TDD-pathogen	0.826	-0.011	-0.037, 0.013	-0.903	-0.011	0.367
	TDD-moral	4.709	0.016	0.002, 0.032	2.170	0.016	**0.031**
	TDD-sexual	0.031	0.001	-0.018, 0.022	0.177	0.001	0.859
	BODS-internal	0.299	0.035	-0.091, 0.162	0.547	0.035	0.585
	BODS-external	0.002	0.004	-0.176, 0.184	0.184	0.003	0.967
	gender	0.723	0.155	-0.204, 0.514	0.850	0.155	0.396

Table 5: *A linear regression testing the relationship between disgust domains and social acceptability self-sniffing frequency controlled for gender. Df is 1 for all variables and 194 for residuals. Statistically significant associations are marked in bold.*

3.5. Association between intimate self-inspection and the health score, hygiene habits, and disgust

A linear regression ($F(5,194) = 14.8$, $p < 0.001$, $R^2 = 0.277$) showed a statistically significant positive association between intimate self-inspection, hygiene-related behaviour, and gender. Respondents

who reported a higher frequency of sniffing own private body areas reported also less hygienic habits. Aside from that, men reported sniffing their private areas more than women did (see Table 6).

Characteristic	Predictors	F	β	95% CI (LL, UL)	t	SE	p
Intimate self-inspection	Health score	0.033	0.002	-0.013, 1.303	0.182	0.007	0.856
	Hygienic behaviour	6.361	-0.519	-0.926, -0.113	-2.522	0.206	**0.012**
	TDDS	0.059	0.001	-0.007, 0.009	0.244	0.004	0.807
	BODS	0.626	-0.047	-0.164, 0.070	-0.791	0.059	0.430
	Gender	44.941	1.01	0.710, 1.303	6.704	0.150	<**0.001**

Table 6: *A linear regression testing the relationship between hygienic behaviour, health score, disgust sensitivity (TDDS and BODS) and Intimate self-inspection frequency controlled for gender. Df is 1 for all variables and 194 for residuals. Statistically significant associations are marked in bold.*

To test for a possible effect of the TDDS and BODS disgust domains, we employed a second linear regression ($F(6,194) = 11.6$, $p < 0.001$, $R^2 = 0.263$), which showed no effect of either disgust domain, but once again showed that the frequency of intimate self-inspection is affected by gender (see Table 7).

Characteristic	Predictors	F	β	95% CI (LL, UL)	t	SE	p
Intimate self-inspection	TDD-pathogen	0.596	-0.008	-0.031, 0.013	-0.772	0.011	0.441
	TDD-moral	0.023	0.001	-0.012, 0.014	0.151	0.006	0.880
	TDD-sexual	0.595	-0.006	-0.025, 0.010	-0.772	0.009	0.441
	BODS-internal	2.905	-0.096	-0.207, 0.015	-1.705	0.056	0.090
	BODS-external	0.931	0.077	-0.081, 0.235	0.956	0.080	0.336
	gender	39.161	0.998	0.683, 1.313	6.258	0.159	**<0.001**

Table 7: *A linear regression testing the relationship between disgust domains and Intimate self-inspection frequency controlled for gender. Df is 1 for all variables and 194 for residuals. Statistically significant associations are marked in bold.*

3.6. Association between cosmetic self-inspection and the health score, hygiene habits, and disgust

A linear regression ($F(5,194) = 1.33$, $p = 0.254$, $R^2 = 0.033$) showed no effect of the self-reported health score, hygiene behaviour, or disgust sensitivity on cosmetic self-inspection (see Table 8).

Characteristic	Predictors	F	β	95% CI (LL, UL)	t	SE	p
Cosmetic self-inspection	Health score	0.069	0.002	-0.013, 0.018	0.264	0.008	0.792
	Hygienic behaviour	2.284	0.355	-0.108, 0.819	1.512	0.235	0.132
	TDDS	0.251	0.002	-0.007, 0.012	0.501	0.005	0.617
	BODS	0.746	-0.058	-0.192, 0.075	-0.864	0.067	0.389
	Gender	1.303	-0.195	-0.533, 0.142	-1.142	0.171	0.255

Table 8: *A linear regression testing the relationship between hygienic behaviour, health score, disgust sensitivity (TDDS and BODS) and cosmetic self-inspection frequency controlled for gender. Df is 1 for all variables and 194 for residuals. Statistically significant associations are marked in bold.*

Furthermore, in the second linear regression ($F(6,194) = 0.74$, $p = 0.615$, $R^2 = 0.022$) we again found no effect of the disgust domain of TDDS or BODS on cosmetic self-inspection (see Table 9).

Characteristic	Predictors	F	β	95% CI (LL, UL)	t	SE	p
Cosmetic self-inspection	TDD-pathogen	0.109	0.004	-0.021, 0.029	0.330	0.012	0.742
	TDD-moral	0.162	0.003	-0.012, 0.018	0.402	0.007	0.688
	TDD-sexual	0.758	0.008	-0.011, 0.029	0.871	0.010	0.385
	BODS-internal	0.002	0.003	-0.123, 0.130	0.053	0.064	0.958
	BODS-external	0.312	-0.051	-0.230, 0.128	-0.561	0.091	0.575
	gender	1.194	-0.198	-0.556, 0.159	-1.093	0.181	0.276

Table 9: *A linear regression testing the relationship between disgust domains and cosmetic self-inspection frequency controlled for gender. Df is 1 for all variables and 194 for residuals. Statistically significant associations are marked in bold.*

4. DISCUSSION

The present study investigated the frequency of self-sniffing of particular body parts and worn clothes as well as possible evolutionary functions of this behaviour. By using a principal component analysis, we have identified three main axes of self-sniffing: social acceptability self-inspection, intimate self-inspection, and cosmetic self-inspection. Subsequently, we compared female and male responses regarding the frequency of self-sniffing along these axes. We found no gender differences in the frequency of social acceptability self-inspection, but men reported more frequent intimate self-inspection, while women more frequent cosmetic self-inspection. Finally, we investigated whether self-sniffing may play a role in monitoring one's health and hygiene status. We found that individuals

who engaged more frequently in social acceptability self-inspection (sniffing body parts such as the armpits, breath, or own worn clothes) reported more frequent health issues. Further, respondents with lower standards of hygiene habits tended to engage significantly more in intimate self-inspection (sniffing body parts such as genitals, anal area, and navel). Finally, cosmetic self-inspection (sniffing body parts such as hands, hair, or neck via hand) was not predicted by the health score, hygiene habits, or disgust sensitivity.

The descriptive part of our study revealed that the most frequently sniffed part were the hands (M = 4.94 -> several times a week) and worn T-shirts (M = 4.89 -> several times a week). This is in line with study by Perl and colleagues (2020), who reported that app. 90% respondents sniff their hands and shirt and study by Li and colleagues (2022a), who showed that app. 85% of respondents sniff their hands and worn clothes at least rarely. Nevertheless, a direct comparison with the two previous studies cannot be done because they used a 4-point scale with undefined time ranges (e.g., 'occasionally' and 'often'). We, on the other hand, have used a 7-point scale with defined time ranges such as 'once a week' and 'every day'. We found the use of defined time range advantageous because i) it provides an insight into the absolute frequency while undefined time range allows only for a relative comparison between variables, ii) it is more concrete and diminishes response noise (it is cognitively less demanding to think of behaviour that occurs once a week than of a behaviour that takes place occasionally), and iii) it is less prone to systematic under- or over-estimation, since for instance 'often' can mean different things to different people (Menold & Bogner, 2016). Moreover, both of the abovementioned studies used inventories that include items not only on self-sniffing but also items on sniffing others, for instance family members, friends, or even strangers. Available evidence, however, suggest that self-sniffing and sniffing others may well be two relatively independent issues. The factor structure of the BOSQ shows that items on sniffing others form a separate factor (the 'Other's body odor' factor). Furthermore, own body odour and the body odour of others induce different levels of disgust, which could significantly influence the willingness to sniff others (Liuzza et al., 2017).

To find out whether the Self-sniffing inventory is a unidimensional or multidimensional measure, we have performed a PCA, which showed that the inventory captures three dimensions of self-sniffing behaviour: i) Social acceptability self-inspection, which includes the sniffing of own body parts and worn clothes, such as T-shirts, armpits, feet, and breath. These body parts and worn clothes are expected to be clean in society as they can be easily perceived, which is why any bad smell would be considered unacceptable. ii) Intimate self-inspection, which includes private body parts such as the genitals, the anus, the navel, and ears. Sniffing these areas is, in Western societies, viewed as inappropriate and might be considered as social taboo. iii) Cosmetic self-inspection pertains to those

body parts which are often linked to good hygiene and the use of fragrances, such as hair, neck, or hands.

This three-dimensional structure indicates that self-sniffing behaviour is not uniform across various body parts. In other words, frequent sniffing of own hands does not necessarily imply frequent sniffing of own armpits. Moreover, this structure also indicates that the evolutionary functions of self-inspection of different body parts may vary and that sniffing of different parts of own body might provide specific information.

We found statistically significant differences in self-sniffing between the genders. We predicted that women would sniff themselves more often, because they tend to place higher value on olfaction and odours in various contexts (Havlicek et al., 2008; Herz & Cahill, 1997, Herz & Inzlicht, 2002). It has also been shown that women sniff their body odour more often than men do (Li et al., 2022a,b). On the other hand, another study reported that the frequency of self-sniffing does not differ that much between men and women (e.g., 96% of women and men sniff their own hands and 92% of both genders sniff own armpits) but men reported more frequent sniffing of their hands after touching their crotch than women did (74% of men vs. 56% of women) (Perl et al., 2020).

The gender differences vary depending on the body area, which is what we had originally partly predicted. Social acceptability self-inspection was reported by both genders similarly often, but men engaged in intimate self-inspection significantly more often than women did. It remains unclear whether women tend to sniff their private areas less often or whether are not willing to admit to doing so because it is considered a social taboo. Women are more likely than men to follow social norms (Bond & Smith, 1996; Eagly & Carli, 1981), which may have influenced their willingness to admit to such behaviour or engage in it. On the other hand, women reported significantly more frequent sniffing of body areas covered by cosmetic self-inspection, which might be an act of taking comfort, self-soothing, or a way of enjoying used fragrances as mentioned above. Various fragrances interact with body odour and humans tend to choose perfumes according to their genetic makeup (Havlíček & Roberts, 2012). Sniffing own odour mixed with a favourite fragrance may thus lead to a strengthening of the sense of self and function as a subconscious reassurance of self (Perl et al., 2020). It may also help reduce stress and anxiety and have a calming effect. Finally, women may have a higher tendency to control the state of their fragrance, because for example ‘stale’ cosmetic products can lose their original odour or even acquire an unpleasant smell, which would be again socially unacceptable.

In our study, we found no statistically significant effect of age on any of the dimensions of self-sniffing behaviour but we found two trends: older people tended to engage less in social acceptability self-inspection and more in intimate self-inspection. This negative tendency to smell the ‘more public’

odours less with increasing age is in line with the findings of a previous study (Li et al., 2022a). It seems that younger people tend to monitor themselves more often: perhaps because they are more involved in mate choice. Then there is also the less likely explanation linked to age-related changes in olfactory sensations (Kaneda et al., 2000), but it should be noted that our participants were between 18 and 38 of age, making it most unlikely that we would have detected any such changes since they are likely to take place at a more mature age. Future studies should recruit more age-diverse groups of participants to explore this issue.

Social acceptability self-inspection is related to those body parts (and worn clothes) which are often checked when one interacts with other people, because they are linked to possible unpleasant body odour that can be perceived by others (Havlíček et al., 2017). We found no significant association between social acceptability self-inspection and hygienic behaviours. It would thus seem that the primary function of this dimension is not to check overall hygiene status but rather just to monitor whether one emits some offensive odour. Another possible function of social acceptability self-inspection dimension may consist in monitoring own health. As predicted, we found a positive association between self-reported health issues and social acceptability self-inspection score. It has been previously reported that people can discriminate between body odour samples collected from individuals previously injected with lipopolysaccharide (bacterial endotoxin leading to a transient systemic inflammation) and healthy individuals (Olsson et al., 2014). To the best of our knowledge, though, no study as yet focused on discrimination between own body odour during illness and in full health. Nevertheless, one can suppose that self-sniffing may serve as an initial check of own health status. Additionally, individuals who suffer from various health issues may be more aware of changes in their body odour, for example the smell of their breath, since they engage in this activity more frequently. This recognition of association between own illness and alteration of own body odour may be another function of the behavioural immune system, which can thus lower the likelihood of pathogen transmission when an individual is socially distancing.

At least in the Western culture, to sniff own private areas is considered quite inappropriate in public, which is why intimate self-inspection is probably limited to private settings. Regardless of this apparent limitation, people tend to engage in this behaviour relatively often. It has been proposed that genital odours have a communicative function in the sexual context (Havlíček & Lenochova, 2008), because for instance the intensity and pleasantness of vaginal odour changes during the menstrual cycle (Doty et al., 1975). This indicates that genital odours carry information about women's reproductive status. We did not measure sexual desire but Li and colleagues (2022b) had reported that individuals who engage more in self-sniffing and place more importance on olfaction also report a higher level of sexual desire. This may have an impact especially on the intimate self-inspection. Moreover, self-sniffing can

be linked to monitoring for a possible malodour before more intimate encounters. Nevertheless, it is still an activity that can considered unhygienic, which may help explain why men who reported higher levels of intimate self-inspection were also less disgusted by their own body odour and overall displayed a lower level of disgust. Moreover, respondents who reported lower levels of hygiene habits might compensate for it by increased intimate self-inspection, because these two were negatively associated.

Monitoring of own body odour from hands operationalized as face-touching is the most often studied self-sniffing type of behaviour in both primates (Dimond & Harries, 1984) and humans (Furmin et al., 2015; Roberts et al., accepted). It can be viewed as easy way of 'collecting' odour on own hand from the body areas which cannot be sniffed directly. One may also carry odours from the environment on one's hands. Besides this examining function, hands are often washed or cream is applied to them. We can sniff the fragranced product on our skin, which may be similar to our hair, as part of cosmetic self-inspection. Moreover, own body odour may also have a calming or soothing effect and it its easily accessible via hands and hair (if the hair is long enough). Enjoyment of the fragrance elements may be an important part of the cosmetic self-inspection, but it need not be necessarily linked to overall hygiene habits. Future studies should ask respondents about the importance of perfumes and other fragrances in their daily life, which might influence some dimensions of the self-sniffing behaviour.

4.1. Limitations

The present study relied on self-reports, which can be a limitation if participants are not fully aware of the behaviour in question and/or if there is social desirability bias. Both issues might be relevant for this study and we do not know to what extent they may have affected the data we had collected. Direct observation might be a more valid approach in case of behaviours included in the social acceptability and cosmetic self-inspection, because those are not considered a social taboo. For intimate self-inspection, however, self-reporting seems to be the only ethical way of exploring this type of behaviour. In the near future, automated biosensors that would monitor self-sniffing behaviour might overcome these constrains.

Another possible limitation is that our sample was relatively homogenous in terms of the age structure and around two third of participants were university students. A previous study found that the frequency of self-sniffing behaviour decreases with age (Li et al., 2022a): we found no effect of age because most of the participants were young adults. Future studies should therefore recruit more socially and age diverse sample.

5. CONCLUSIONS

Our results show that humans engage in self-sniffing behaviour quite frequently, but the frequency varies considerably between individuals and between particular body parts. The self-sniffing inventory we had developed captures three dimensions: social acceptability self-inspection, intimate self-inspection, and cosmetic self-inspection. The pattern of associations with health issues, hygiene habits, and disgust sensitivity indicates that each dimension might serve a different evolutionary function. Social acceptability self-inspection was positively associated with health issues, while intimate self-inspection was negatively associated with hygiene habits and gender.

Future studies should investigate the contexts in which olfactory self-inspection takes place. This may provide further insights into why are humans sniffing themselves. Finally, cross-cultural studies on olfactory self-inspection might show why some respondents sniff certain some body parts more frequently, as indicated in the study of Li and colleagues (2022) which compared Chinese and US respondents. It could also shed some light on whether this behaviour is influenced by local social taboos.

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Data availability

The data associated with this research are available at https://osf.io/vj598/.

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Statement of contribution

This is to confirm that Mgr. Dagmar Schwambergová significantly contributed to the following publication:

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She contributed to the conceptualisation, investigation, methodology, data analysis, visualisation, manuscript writing and subsequent editing and revisions.

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

_____

doc. Mgr. Jan Havlíček, Ph.D.
supervisor

Chapter 15

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

Vít Třebický [a,b,c,*], Sylvain Delplanque [d], Camille Ferdenzi [e], Bernhard Fink [f,k], Lucie Jelínková [c], Žaneta Pátková [c], S. Craig Roberts [g], Susanne Röder [h], Tamsin K. Saxton [i], Dagmar Schwambergová [c], Zuzana Štěrbová [c,j], Jitka Třebická Fialová [c], Jan Havlíček [c]

[a] Faculty of Physical Education and Sport, Charles University, Prague, Czech Republic
[b] National Institute of Mental Health, Klecany, Czech Republic
[c] Faculty of Science, Charles University, Prague, Czech Republic
[d] Swiss Center for Affective Sciences, University of Geneva, Geneva, Switzerland
[e] Lyon Neuroscience Research Center, Bron, France
[f] Department of Evolutionary Anthropology, University of Vienna, Biosocial Science Information, Biedermannsdorf, Vienna, Austria
[g] Division of Psychology, University of Stirling, Stirling, United Kingdom
[h] Department of Psychiatry, Sozialstiftung Bamberg, Bamberg, Germany
[i] Department of Psychology, Northumbria University, Newcastle, United Kingdom
[j] Faculty of Arts, Charles University, Prague, Czech Republic
[k] Human Evolution and Archaeological Sciences (HEAS), University of Vienna, Vienna, Austria

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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;

* Corresponding author at: Faculty of Physical Education and Sport, Charles University, José Martího 31, 162 52 Prague 6, Czech Republic.
E-mail address: vit.trebicky@ftvs.cuni.cz (V. Třebický).

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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 $\geq$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 $\leq$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.

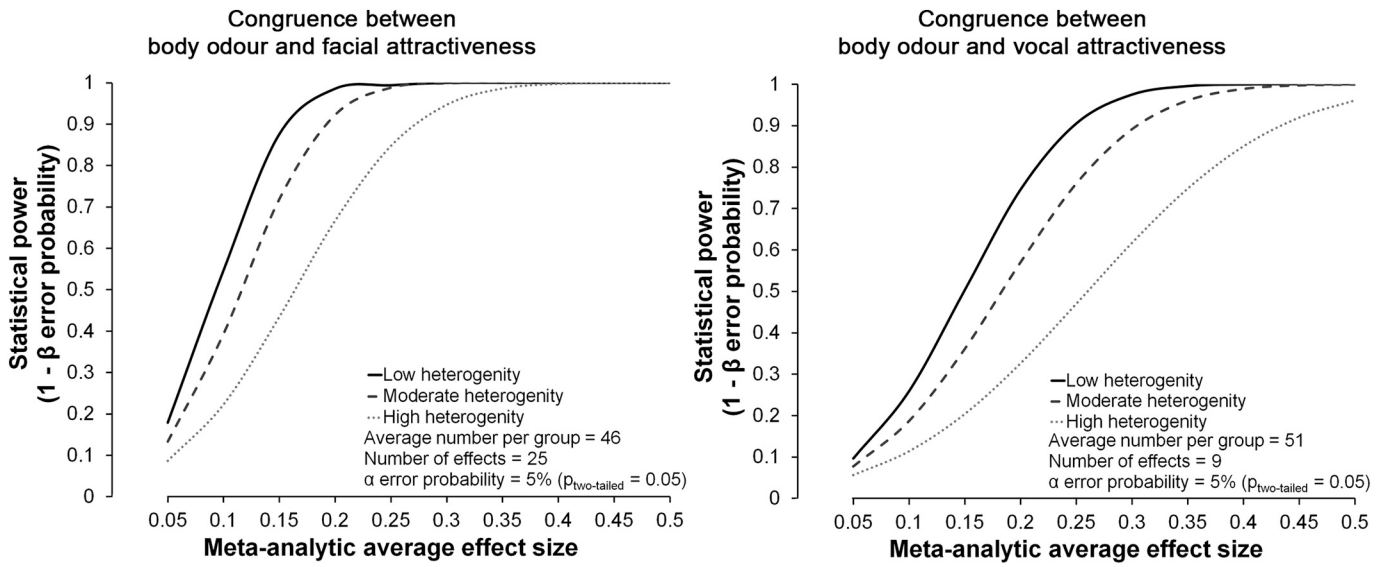

Fig. 1. Power curves for the sensitivity to detect meta-analytic effects as a function of heterogeneity. The plots display the sensitivity analysis for the meta-analysis of congruence between body odour and facial attractiveness (left panel) and between body odour and vocal attractiveness (right panel). 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] 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.

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

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

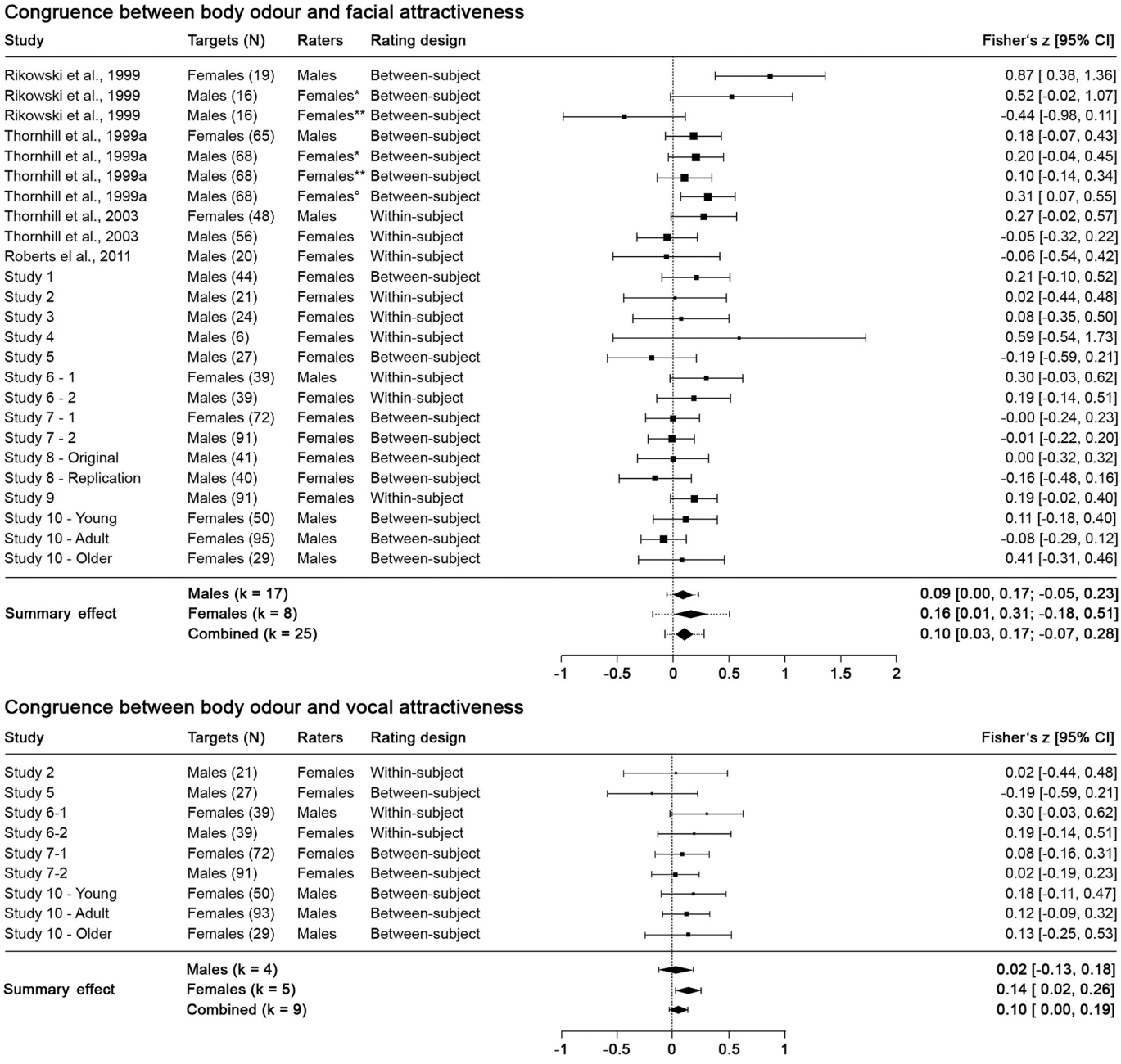

Fig. 2. Forest plots for congruence meta-analyses. Squares represent weighted mean effects of individual studies, and error bars their 95% confidence intervals. Diamonds represent summary effects, their width the 95% CIs, and dashed error bars their 95% PIs. *Female raters in fertile, **non-fertile phase 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.

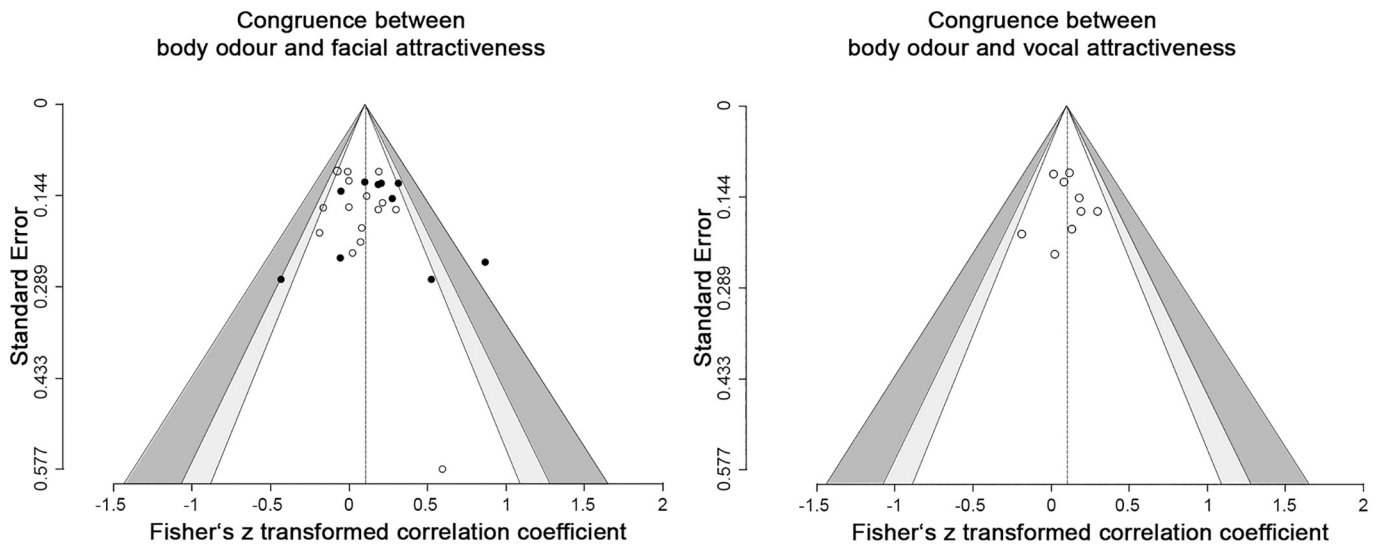

Fig. 3. Funnel plots for congruence meta-analyses. Area outside the contour-enhanced funnels represent *p* values <0.01, dark grey areas p values between 0.01 and 0.05, light grey p values between 0.05 and 0.1, and areas inside the funnel p values >0.1. Full circles illustrate published and empty circles 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

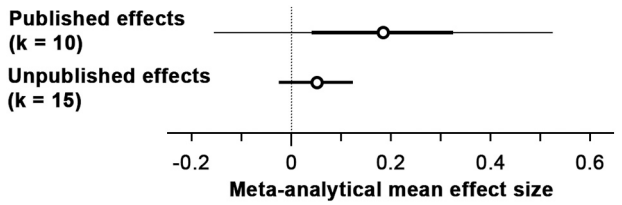

Fig. 4. Comparison of meta-analytic averages between published and unpublished effects. Circles represent mean effects. Thick error bars their 95% CI and thin error bars 95% PI. Due to observed heterogeneity only in the published effects, the mean effect is accompanied by 95% PI. The plot was generated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

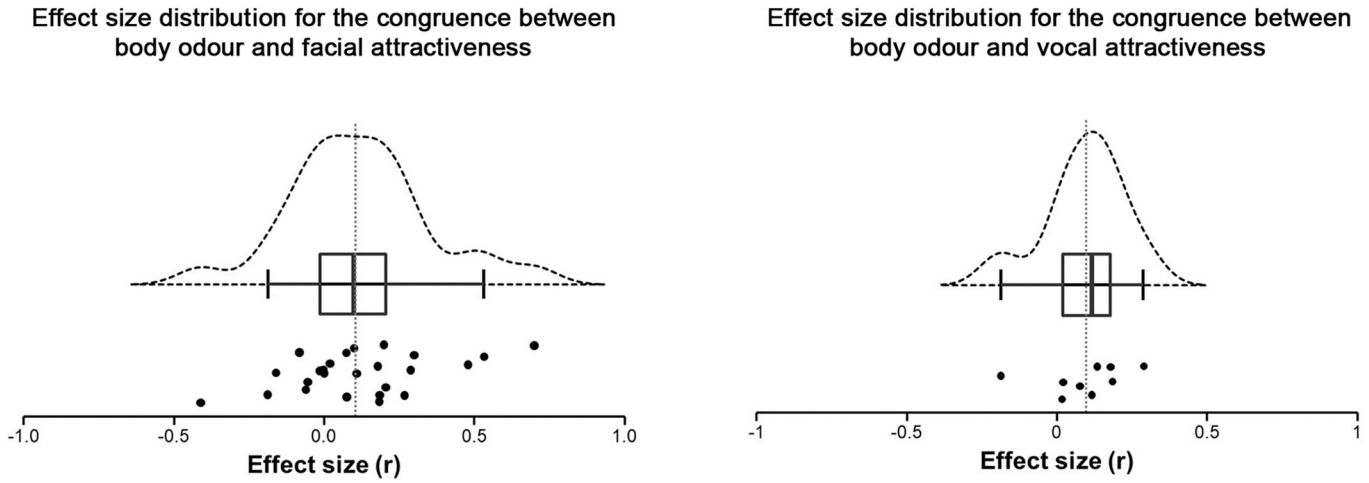

Fig. 5. Raincloud plots for effect size distribution. Density plots show effect sizes distribution, boxplots show median (thick line), 25th and 75th percentile (interquartile range, box), and minimum and maximum (error bars); jittered dots represent individual effect sizes; dotted vertical line shows 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 $p_{two\text{-}tailed}$ 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

Vít Třebický: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project

administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Sylvain Delplanque:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Camille Ferdenzi:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Bernhard Fink:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Lucie Jelínková:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Žaneta Pátková:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **S. Craig Roberts:** Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Susanne Röder:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Tamsin K. Saxton:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Dagmar Schwambergová:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Zuzana Štěrbová:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Jitka Třebická Fialová:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. **Jan Havlíček:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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

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Statement of contribution

This is to confirm that Mgr. Dagmar Schwambergová significantly contributed to the following publication:

Třebický, V., Delplanque, S., Ferdenzi, C., Fink, B., Jelínková, L., Pátková, Ž., Schwambergová, D., ... & Havlíček, J. (2022). 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. *Evolution and Human Behavior*.

She contributed to the investigation, writing of the manuscript methodology and subsequent editing and revisions.

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

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doc. Mgr. Jan Havlíček, Ph.D.
supervisor

CONCLUSIONS

The thesis consists of two parts. The first part provides an outline of the theoretical background for a better understanding of the chapters in the following, second part. It introduces the behavioural immune system and its functioning and, subsequently, also the olfaction-mediated behavioural defence as one part of this system. It points to possible future directions of research in this area and draws attention to various shortcomings and lacunae. The second part of the thesis consists of nine chapters (three reviews and six experimental studies), which may at first appear heterogeneous. A closer look, however, reveals that the central topic is olfaction and behavioural immune defence mechanisms, especially in the context of partner selection, although this system also works in various contexts other than mate choice. The aim of the introductory part of the thesis is to connect these studies by highlighting the questions they aim to answer.

At the beginning of the overview of literature, I present a theory of the behavioural immune system (BIS) and its functioning and outline how it is connected to the physiological immune system. This it is followed by a presentation of the methodology used in the BIS and disgust research, based on which I introduce a 'naturally occurring experiment' during the pandemic of coronavirus. Further, I investigate the role of olfaction in the abovementioned defence mechanisms and its specific benefits. As I repeatedly note, pathogen avoidance induced by BIS activity is not limited to the context of mate choice but majority of research focuses on this context and consequently most available data have been collected from this perspective. In introducing these studies, I highlight the second important part of mate choice, namely choosing a partner with high-quality immune system that can be passed to the offspring. In olfactory research, few studies so far studied this issue, so I extended the overview by some thoughts on the association between olfaction and other modalities.

Interestingly, it seems that every modality carries different information about an individual, which is what the 'multiple messages' hypothesis suggests. Finally, in light of our one of the last studies, I speculate about yet another function of the BIS, namely its possible role in the perception of own health status and its changes, which could facilitate a withdrawal from own social group to protect others, including relatives. In this first part of the thesis, I raise many questions which I would like to investigate in the future.

In the second part of the thesis, I follow the direction outlined in the preceding theoretical background. I start with a review of the BIS that goes beyond the outline from the theoretical part of the thesis and emphasises its social implications and links to obsessive behaviours. Subsequently, I introduce a paper in which we showed that respondents did not report a higher level of pathogen disgust during the first wave of Covid-19 than during the period when all restrictions were lifted in the summer 2021, which is what one would have expected with activated BIS. In this paper, I list various factors which could affect the activation of this system. While Chapter 7 introduces the BIS in general, Chapter 10 focuses on olfaction-mediated pathogen avoidance. It shows that many factors, including familiarity or dominance, influence the resulting avoidant behaviour (and thus also reproductive success). The following empirical study investigates alterations in body odour, facial, and vocal attractiveness after immunoactivation by vaccination. We found that two weeks after vaccination, body odour samples were rated as more attractive while facial photographs were rated as less attractive. This may indicate that information carried by different modalities might persist for different lengths of time.

There exists a substantial body of literature on the importance of olfaction in choosing a healthy partner but in the following study, we aimed at the indirect benefits provided by partner, in particular the 'good' genes inherited by the offspring. We wanted to see whether body odour provides cues to the functioning of the immune system, which is what has been shown by facial research. In this case, though, we found no association between the levels of antibodies after vaccination and body odour quality, nor a relationship between the innate and adaptive immunity and body odour quality. We also looked at the data from the same project but with facial attractiveness and healthiness as the main variables and, once again, we found no significant associations between vaccination-induced antibody levels and perceived facial attractiveness or healthiness. We also found no significant association between antibody levels and facial colouration. These results run contrary to previous studies.

The last two empirical studies focused on the role of olfaction in relationships and self-inspection. The first is a qualitative study mapping the role of olfaction in various situations in a relationship context. We found, for example, that if men are ill, their female partners can identify a change in their body odour, which they then perceive as more unpleasant. Subsequently, we showed that olfactory self-inspection is not unidimensional but breaks into three dimensions, each with a possibly different function, whereby people sniff different parts of their bodies to acquire different information about themselves. Because some studies have

reported close connections between attractiveness ratings of faces, voices, and body odour, in another review with a meta-analysis we wanted to test the strength of associations between the abovementioned ratings. We found only a weak association between these variables, which supports the ‘multiple messages’ hypothesis.