Proactive immune responses: physiological and behavioral mechanism, influences and triggers

Dissertation

Zur Erlangung der Würde des Doktors der Naturwissenschaften des Fachbereichs Biologie der Fakultät für Mathematik, Informatik und Naturwissenschaften

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Vorgelegt von
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List of Publications

1. Disease-related Disgust promotes antibody release in human saliva (Judith K. Keller, Clemens Wülfing, Jannes Wahl, Esther K. Diekhof)
   The study design was conceptualized in cooperation of J.K.K. and E.D. J.K.K. accumulated the stimulants, trained and managed three Bachelor students (among others J.W.) during data collection. JK analyzed the data in constant consultation with E.D., wrote the first version of the manuscript, and further edited it according to E.D., J.W. & C.W. feedback.

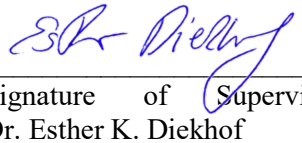
2. SARS-CoV-2 specific sIgA in saliva increases after disease-related video stimulation (Judith K. Keller, Alex Dulovic, Jens Gruber, Johanna Griesbaum, Nicole Schneiderhan-Marra, Clemens Wülfing, Jana Kruse, Annika Hartmann & Esther K. Diekhof)
   E.D & J.K.K. initiated the research and further set the study conception, design and material. A.H., J.K., and J.K.K. collected the data. A.D. designed and supervised the laboratory measurements of salivary antibodies. A.D., Je.G. & Jo. G performed the laboratory analyses. J.K.K. & E.D. statistically analyzed the data. N.S.M. obtained funding. J.K.K. wrote the first draft of the manuscript with the support of E.D. All remaining authors provided feedback on the manuscript draft.

3. Influence of female sex hormones on proactive behavioral and physiological immune parameters (Judith K. Keller & Esther K. Diekhof)
   E.D. initiated the research and further set the study conception, design, and material. J.K.K. managed and conducted part of the data collection. J.K.K. statistically analyzed the data, wrote the first version of the manuscript, and further edited it according to E.D.'s feedback.

4. Visual Cues of Respiratory Contagion: Their Impact on Neuroimmune Activation and Mucosal Immune Responses in Humans (Judith K. Keller & Esther K. Diekhof)
   E.K.D acquired funding, conceptualized and designed the study, and supervised the project. J.K. contributed to the design of the study, programmed the experiment, performed data acquisition and formal analysis. E.K.D. reviewed the results, and both J.K. and E.K.D. wrote the manuscript.

25.04.2025 Hamburg

Date, Place



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I. Abstract

The well-known reactive immune system responds to pathogens that come into contact with the host. However, this reactive response comes at a high cost, such as inflammation that weakens the body. Given the high selection pressure, it would be reasonable to conclude that further proactive mechanisms have developed. This cumulative dissertation investigates the physiological, behavioral, and neural underpinnings of such proactive immune responses, particularly focusing on mucosal immunity as measured in secretory immunoglobulin A (sIgA) in saliva. Across four studies, we examined how visual respiratory disease cues influence sIgA secretion and explored potential moderators, including hormonal states and neural processing. In Chapter I, we developed and validated a video-based experimental paradigm to elicit disease-related disgust, reliably triggering increased sIgA levels. Chapter II extended these findings by demonstrating that exposure to respiratory disease cues elicited a general sIgA response and elevated antigen-specific sIgA levels against SARS-CoV-2. In Chapter III, we investigated the potential influence of the menstrual cycle on the magnitude of the sIgA response to respiratory disease cues. Contrary to our expectations, no systematic modulatory effects of estradiol or progesterone on the proactive immune responses were observed, indicating that they may be hormonally stable across the menstrual cycle. Finally, in Chapter IV, we used functional magnetic resonance imaging to identify the neural correlates of proactive immune activation. Exposure to visual respiratory disease cues activated regions commonly implicated in threat and disgust processing, including the anterior insula and amygdala. Moreover, the strength of activation in the anterior insula was positively associated with the sIgA response, pointing to a functional neural-immune link in proactive defense.

Together, these studies provide converging evidence for the existence and specificity of proactive immune responses in healthy humans.

These findings open a range of promising directions for future research. A key next step

involves deepening our understanding of the underlying mechanisms in healthy individuals. Moreover, the experimental paradigm developed in this thesis, which reliably elicited both mucosal and behavioral immune responses to visual respiratory disease cues, offers a valuable tool for translational research. It could be applied to study altered immune reactivity in patients, including individuals with behavioral immunity or physiological immune function impairments, thereby contributing to a more nuanced understanding of immune dysregulation.

II. Zusammenfassung

Das bekannte reaktive Immunsystem reagiert auf Krankheitserreger, die mit dem Wirt in Kontakt kommen. Diese reaktive Immunantwort ist jedoch mit hohen Kosten verbunden, wie z. B. entzündlichen Prozessen, die den Körper schwächen können. Angesichts dieses hohen Selektionsdrucks erscheint es plausibel, dass sich zusätzlich proaktive Mechanismen entwickelt haben. Die vorliegende kumulative Dissertation untersucht die physiologischen, behavioralen und neuronalen Grundlagen solcher proaktiven Immunantworten, mit einem besonderen Fokus auf die mukosale Immunität, gemessen über sekretorisches Immunglobulin A (sIgA) im Speichel. In vier Studien wurde untersucht, wie visuelle Reize mit Bezug zu respiratorischen Erkrankungen die sIgA-Sekretion beeinflussen, und ob hormonelle Zustände sowie neuronale Verarbeitungsprozesse diese Antwort moderieren.

In Kapitel I wurde ein videobasiertes Experimentierparadigma entwickelt und validiert, dass gezielt ekelbezogene Reaktionen hervorruft und zuverlässig eine Erhöhung der sIgA-Werte auslöst. Kapitel II erweiterte diese Befunde, indem gezeigt wurde, dass visuelle Reize mit Bezug zu respiratorischen Erkrankungen nicht nur eine generelle sIgA-Antwort auslösen, sondern auch zu einem Anstieg von antigen-spezifischem sIgA gegen SARS-CoV-2 führen. In Kapitel III wurde der Einfluss des Menstruationszyklus auf die Stärke der sIgA-Antwort untersucht. Entgegen unserer Annahmen konnten keine systematischen modulierenden Effekte von Östradiol oder Progesteron festgestellt werden, was auf eine hormonelle Stabilität der proaktiven Immunantworten im Verlauf des Zyklus hindeutet. In Kapitel IV wurden mittels funktioneller Magnetresonanztomographie (fMRT) die neuronalen Korrelate proaktiver Immunaktivierung identifiziert. Die Konfrontation mit visuellen Reizen zu respiratorischen Erkrankungen aktivierte Gehirnregionen, die typischerweise mit der Verarbeitung von Bedrohung und Ekel assoziiert sind, darunter die anteriore Insula und die Amygdala. Zudem korrelierte die Stärke der Aktivierung in der anterioren Insula positiv mit der sIgA-Antwort,

was auf eine funktionelle Verbindung zwischen neuronalen und immunologischen Prozessen im Rahmen proaktiver Abwehrmechanismen hinweist.

Insgesamt liefern diese Studien konsistente Hinweise auf die Existenz und Spezifität proaktiver Immunantworten bei gesunden Menschen. Die Ergebnisse eröffnen vielfältige Perspektiven für zukünftige Forschung. Ein zentraler nächster Schritt besteht darin, die zugrunde liegenden Mechanismen bei gesunden Individuen weiter zu entschlüsseln. Darüber hinaus stellt das in dieser Arbeit entwickelte Experimentierparadigma, dass zuverlässig mukosale und behaviorale Immunreaktionen auf visuelle Reize respiratorischer Erkrankungen auslöst, ein wertvolles Instrument für die translationale Forschung dar. Es könnte verwendet werden, um veränderte Immunreaktionen bei Patient*innen mit Einschränkungen im Bereich der behavioralen oder physiologischen Immunantworten zu untersuchen und somit zu einem differenzierteren Verständnis immunologischer Dysregulation beitragen.

III. Abbreviations

BIS	Behavioral immune system
BOLD	Blood oxygenation level-dependent
COVID-19	Coronavirus disease 2019
ELISA	Enzyme-linked immunosorbent assay
fMRI	Functional magnetic resonance imaging
GLM	General Linear Model
H-chain	Heavy chain (in immunoglobulin)
Ig	Immunoglobulin
IL	Interleukin
L-chain	Light chain (in immunoglobulin)
NST	Nucleus of the solitary tract
OCD	Obsessive-compulsive disorder
PAG	Periaqueductal grey (PAG)
PIS	Physiological immune system
pIgR	Polymeric-immunoglobulin receptors
pVtD	Perceived Vulnerability to Disease
RBD	Receptor binding domain
Sars-CoV-2	Severe acute respiratory syndrome coronavirus 2
sIgA	Secretory Immunoglobulin A
SPM	Statistical Parametric Mapping
TNF-α	Tumor Necrosis Factor alpha

1. Introduction

1.1 The Immune System as a Reactive System

The pressure of pathogens has led to a continuous arms race between them and their hosts (Siddle and Quintana-Murci, 2014). Heritable defense mechanisms against pathogens can already be found in single-cell organisms; these evolved into complex innate immune systems in multicellular organisms and an additional adaptive immune system in jawed vertebrates (Gnathostomes) (Beutler, 2004). This arms race results in multiple levels of immune defense in humans.

1.1.1 Innate Immune System

The innate immune system serves as a fast first response to entering pathogens that have surpassed outer barriers. It detects pathogens with its germline-encoded receptors that are specialized on highly conserved pathogen-associated components (Pancer and Cooper, 2006) shared by large groups of pathogens. Therefore, the innate immune system detects pathogens without previous contact and then gives an inflammatory response within minutes. This response is partly cellular, i.e., macrophages, dendritic cells, neutrophils, and natural killer cells, as well as humoral, i.e., LPS binding protein, C-reactive protein, and other pentraxins, collectins, and antimicrobial peptides (Turvey and Broide, 2010). This fast but unspecific immune response has its pitfalls. The broad detection of the receptors can lead to defense mechanisms against the host's own or non-harmful tissues. Additionally, the inflammatory immune responses triggered by the innate immune system can be a stressor to neighboring tissue and the whole body (Yatim and Lakkis, 2015). This led to the evolution of a more specific adaptive immune system.

1.1.2 Adaptive Immune System

The adaptive immune system is slower but more specialized. The leading actors are B and T lymphocytes; these express diverse receptors that can recognize rapidly evolving pathogens (Pancer and Cooper, 2006). These receptors are called immunoglobulins (Igs) on B lymphocytes and T-cell receptors on T lymphocytes. They are able to distinguish antigens (structures on the surface of pathogens that the immune system recognizes) from their own tissues, minimizing the risk of damaging them with the immune response (Yatim and Lakkis, 2015). While T-cell receptors are always surface-bound, B lymphocytes can additionally secrete their immunoglobulins (also called antibodies) (Boehm, 2011). These Igs can be sorted into five classes (IgG, IgA, IgM, IgD, and IgE), with IgG and IgA having four and two subclasses, respectively. All Igs consist of two identical heavy chains (H) and two identical light chains (L) bound by disulfide bonds (Delves and Roitt, 2000). The L- and the H-chain have a variable region ending in an N-terminal (also called amino-terminal); together with the first constant region, they are called Fab-fragment, the 'ab' standing for 'antigen-binding.' In IgG, IgA, and IgD, the H-chains have three constant regions, while IgE and IgM have four. The latter two or, respectively, three regions are part of the Fc-fragment; the 'c' stands for 'crystallizable' (also see Fig.1). IgA and IgM further have J-chains (joining chains), allowing them to form dimers and pentamers (Chiu et al., 2019; Mix et al., 2006).

Detection of pathogens by Igs or T-cell receptors leads to an extensive proliferation of lymphocytes. B lymphocytes transform into plasma cells in the following process, producing further Igs, while T lymphocytes differentiate into helper and effector subsets. The helper cells, on the one hand, are regulating and directing the immune response (i.e., production of plasma cells and memory cells); the effector cells (also called cytotoxic T cells), on the other hand, are directly attacking and destroying pathogens (Yatim and Lakkis, 2015). In order to suppress responses against oneself, regulatory T and B cells are formed and restrain excessive inflammatory responses (Josefowicz et al., 2012; Mauri and Bosma, 2012). Most lymphocytes

are eliminated at the end of the immune response, and the surviving ones form long-lived memory lymphocytes (Sprent, 1994). These memory cells facilitate a faster and more effective response if the host ever reencounters the pathogen (Yatim and Lakkis, 2015).

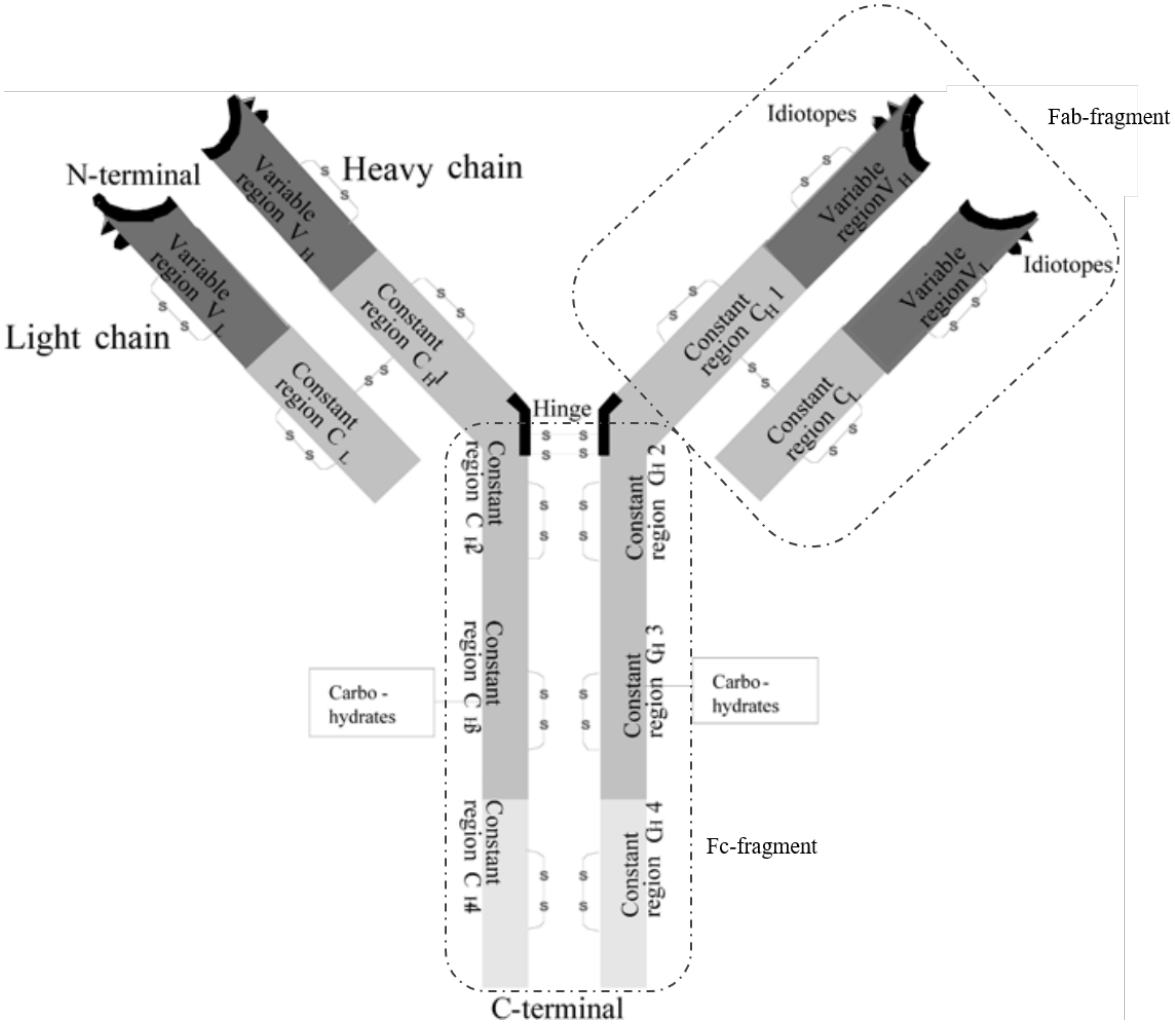

Figure 1: Schematic structure of a monomeric Ig with heavy and light chains, N-terminal, C-terminal, and disulfide bonds. Fab- and Fc-fragment marked by dotted lines, adapted from (Mix et al., 2006).

While the innate and the adaptive immune systems are widely seen as two different systems, research has found that they interact. More specifically, immature dendritic cells of the innate immune system mature under the stress of innate receptors and activate naïve T lymphocytes by sending danger signals to regulatory T lymphocytes (Clark and Kupper, 2005). Cells of the immune system are found throughout the body, i.e., in blood, bone marrow, the spleen, on the skin, in the gut, and on mucosal membranes in the upper respiratory tract, whereby 60-70 % of the lymphocytes in the whole body reside in the mucosal tissues (Farber, 2021; Kurono, 2022).

1.1.3 Mucosal Immune System of the Upper Respiratory Tract

The mucosal immune system can be found in regions that come in contact with foreign particles, most commonly the gastrointestinal tract, the female reproductive tract, and the upper respiratory tract. The latter consists of the nose, nasal cavity, mouth, throat, and larynx. The mucosal immune system in the upper respiratory tract consists of a single layer of epithelium, which serves as a physical barrier, produces mucus, and utilizes ciliated cells to capture and transport particles. This is supported by innate (i.e., anti-microbial peptides, α- and β-defensin) and adaptive (i.e., intraepithelial lymphocytes, immunoglobulins) immune components along with a natural microbiota (Kurono, 2022; McGhee and Fujihashi, 2012).

One of the major players in the adaptive immune support to the epithelial cells is the secretory immunoglobulin A (sIgA). Plasma cells adjacent to the mucosal epithelial cells secrete IgA in a dimeric form bound by J-chains. It is then transported by polymeric-immunoglobulin receptors (pIgR) across epithelial cells (transcytosis). This is granted through the secretory component, a polypeptide, and an extracellular portion of the pIgR, which binds to the IgA (turning it into sIgA) when secreted into the mucosa. The secretory component makes the IgA very stable, protecting it from proteases and acids (Strugnell and Wijburg, 2010). SIgA is constantly secreted into the mucosa at a base rate and can be rapidly upregulated by (para)sympathical (Carpenter et al., 1998) and mechanical stimulation (Proctor and Carpenter, 2001). It plays a key role in several immunological processes, including immune exclusion, where it binds to antigens to prevent their attachment to epithelial cells, and intracellular neutralization, which involves the inhibition of viral replication within epithelial cells. It is key in maintaining homeostasis at mucosal surfaces through antigen excretion (see Figure 2) (Corthésy, 2013; Strugnell and Wijburg, 2010).

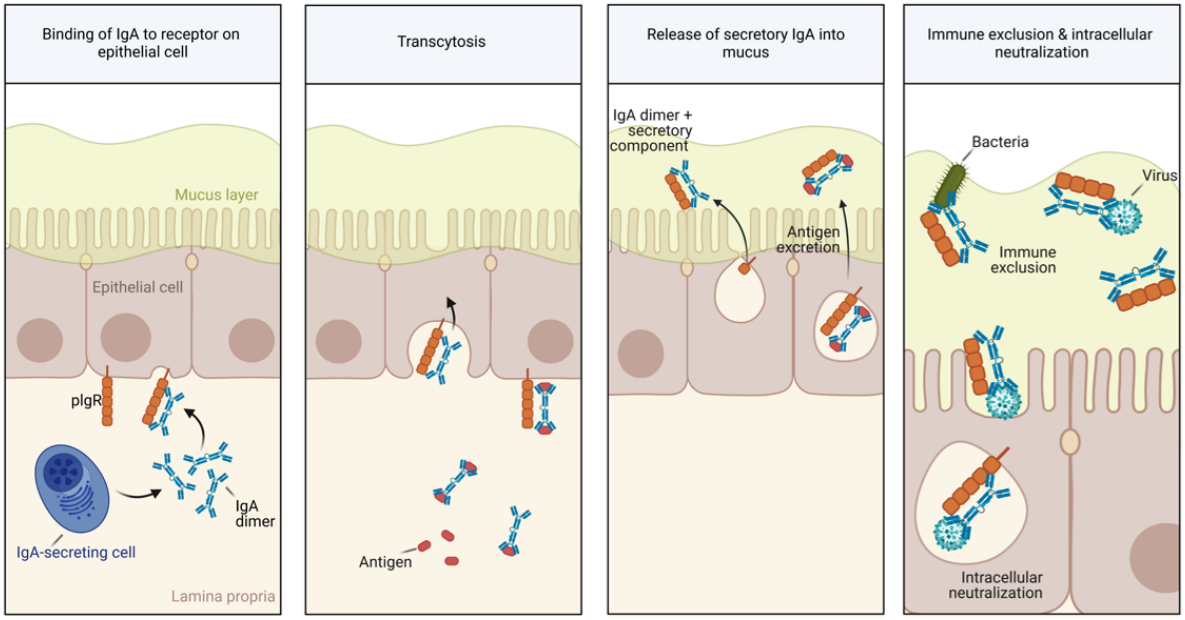

Figure 2: Schematic overview of sIgA transcytosis, immune exclusion, and immune neutralization (generated with biorender.com)

1.2 Proactive Immune Response

These previously described mechanisms are all reactive to pathogens that come into contact with the host. However, this reactive response comes at a high cost, such as inflammation that weakens the body. Given the high selection pressure, it would be reasonable to conclude that further proactive mechanisms have developed.

1.2.1 Detection of Infection Potential

Proactive immune responses of any kind require the ability to detect potentially infectious situations. However, this detection may not always be straightforward. While most people can identify mold on rotten food through visual inspection, most pathogens are microscopic and cannot be seen with the naked eye. It has been suggested that humans evolved a detection system that recognizes predictors of increased contagion probability, assessing certain situations as more or less contagious. For instance, humans or other animals are more likely to carry pathogens that threaten humans than plants. Additionally, their secretions are more likely

to be infectious than other parts, such as their hair (Tybur and Lieberman, 2016). Evidence supporting this theory has been found in a study examining reactions to objects with varying moisture levels. This study revealed that people primarily expect pathogens in items with a moderate moisture level rather than in dry or very moist objects (Iwasa et al., 2020). Understanding which situations and/or locations are more likely associated with pathogens, as well as the visible recognition of a threat, is most likely learned by observing the behavior of others (Fessler and Navarrete, 2003; Stevenson et al., 2010a) and/or through conditioning (Borg et al., 2016; Rozin, 1986).

However, as highly sociable animals, humans would incur a high cost, eliciting proactive immune responses whenever they encounter a conspecific, just because they are more likely to be infectious than plants. Therefore, it is important to recognize infected or sick conspecifics. The ability to do so is evident in many social species, from termites (Cremer et al., 2007) to apes (Poirotte et al., 2017). While humans can detect sickness in conspecifics through body odor (Olsson et al., 2014), their most significant sense in this context is vision, as it enables detection without close contact, thus minimizing the chance of infection. Whenever the reactive immune system is active, the affected person shows specific cues in behavior and appearance. So-called “sickness behaviors”, such as inactivity, sleepiness, reduced appetite, and hygiene, occur when animals and humans need to conserve energy while fighting infections (Hart and Hart, 2019). Humans seem to be able to recognize these kinds of infection cues.

Simple indicators, such as mouth curvature, facial shape related to weight, and color cues signaling general health, are also important (Henderson et al., 2016). In particular, the latter is crucial for recognizing acute sickness in others. Low levels of carotenoids in skin color, which result in a yellower and darker appearance, are linked to aversion due to the perception of lower health (Lefevre et al., 2013; Whitehead et al., 2012). Movement serves as another signal for detecting illness in others. Humans can differentiate between sick and healthy individuals based

on their walking patterns, with sick individuals tending to walk more slowly and rigidly (Hansson et al., 2023). Consequently, humans are inclined to exhibit proactive immune responses when encountering conspecifics who are pale, have dark circles under their eyes, walk slowly and rigidly, and are in environments associated with a higher contagion probability, such as being in bed, surrounded by tissues, or displaying apparent symptoms like sneezing, coughing, or blowing their nose.

1.2.2 Disgust and Behavioral Mechanisms

The concept of a behavioral immune system (BIS) was first introduced by Schaller in 2006. In this article, he acknowledges that the term "psychological immune system" may have been more appropriate for his concept, as it encompasses emotions and cognitions; however, he explains that this term has been previously utilized in another context and, therefore, he retains the use of BIS. This term has since been repeatedly employed and commonly discussed in research over the last two decades (i.e., Ackerman et al., 2018; Bacon and Corr, 2020; Terrizzi et al., 2013). In the original concept of the BIS, Schaller suggests that upon perceiving the potential for infection, specific emotions and cognitions, such as disgust, alongside behaviors like avoidance and social exclusion, are triggered as proactive mechanisms against pathogens (Schaller, 2006).

The core of this theory is the emotion of disgust. It is one of the six basic emotions proposed by Darwin in 1872 and has since become a widely researched emotion. It is defined as a feeling of revulsion that can be linked to nausea and the urge to withdraw from the elicitor of disgust (Rozin et al., 2000). Disgust is often, though not exclusively, associated with specific physiological responses, including a distinctive facial expression characterized by a wrinkled nose and raised upper lip (Pochedly et al., 2012), as well as decreased blood pressure, heart rate, and lower skin conductance (Stark et al., 2006). Notably, the facial expressions associated with disgust are consistent across cultures (Ekman and Friesen, 1971), suggesting that disgust

is rooted in human evolution. The most widely accepted theory regarding its original function is distaste, a repulsive response to ‘bad taste’ (primarily bitterness) to protect the body from toxins entering orally (Rozin et al., 2000). Distaste can also be observed in animals and even in infants (Berridge, 2000; Grill and Norgren, 1978; Steiner, 1973). Disgust likely evolved from this fundamental function to become an emotion that protects us from more than just unpleasant tastes, engaging multiple senses, with infectious diseases probably being the primary driver for its evolution (Curtis et al., 2004).

Current research agrees that there are different types of disgust, each with slightly varying functions; however, these types are defined differently throughout the literature. The most common categories include physical/pathogen disgust, interpersonal/sexual disgust, and moral disgust (Chapman and Anderson, 2012; Tybur et al., 2013). Pathogen disgust is the most significant category regarding behavioral and physiological immune mechanisms, as a wide range of stimuli can trigger it. Additionally, some researchers propose a division into two factors: core disgust and contamination-based disgust. Core disgust includes stimuli that evoke a sense of offensiveness through sight, taste, or smell, such as rotten food, waste products, and small animals (Olatunji et al., 2007b; Rozin et al., 2000). On the other hand, contamination-based disgust is characterized by a reaction to the perceived threat of contagion and is triggered by stimuli like sick individuals, the smell of urine, and contact with others' bodily fluids (Olatunji et al., 2007b). Thus, pathogen disgust is a direct response to detecting cues for potential infections, as previously described. Disgust promotes the avoidance of physical contact with the elicitor, and pathogen avoidance becomes the primary behavioral mechanism in a proactive immune response (Curtis, 2014; Curtis et al., 2011; Oaten et al., 2009). When the literature discusses pathogen avoidance behaviorally, it focuses on actions taken by individuals or groups to reduce their chances of infection (Curtis, 2014). For example, insects (Cremer et al., 2007), sheep (Hutchings et al., 2001), and primates (Philippon et al., 2023) avoid feces in

their environments while foraging and grooming in primates (Silk et al., 2003) can also be considered a pathogen avoidance mechanism. Humans exhibit similar behaviors (Stevenson et al., 2011a), such as having extra rooms and flushable toilets to keep feces out of their lives. In situations of acute disgust, humans generally feel the urge to leave the area. This starts with simply looking away from disgusting materials (Armstrong et al., 2022; Olatunji et al., 2008; Rozin et al., 1999) and can escalate to actually moving away or avoiding any contact (Koch et al., 2002; van Overveld et al., 2010). While there is no definitive proof of humans avoiding conspecifics displaying signs of sickness, preliminary evidence has been found. For instance, humans tend to avoid socializing with conspecifics who appear to have insufficient sleep (Sundelin et al., 2017), likely due to overlapping signs with sick individuals (like dark circles under their eyes). A recent study revealed that participants gazed at images of sick people for shorter durations than they did at images of healthy individuals, and their pupils dilated more when viewing sick faces, which correlated with higher avoidance ratings for those pictures, suggesting threat detection and potential avoidance (Leung et al., 2023). Moreover, if participants viewed a disease-related video before completing an approach and avoidance task with neutral human faces, their avoidance tendencies increased compared to a control group (Mortensen et al., 2010).

The psychological and behavioral mechanisms of the proactive immune response can be costly, potentially causing individuals to miss out on activities, people, and resources that may, aside from being potentially pathogenic, also be beneficial (e.g., in terms of mate choice or food resources) (Tybur et al., 2013). These mechanisms can also become psycho-pathological, leading to psychiatric conditions such as anxiety and obsessive-compulsive disorder (Bhikram et al., 2017; Davey, 2011; Olatunji et al., 2010), which further heighten the costs of disgust and avoidance based on false alarms related to often non-pathogenic stimuli. Therefore, other mechanisms of the proactive immune system are crucial in interacting with these behavioral mechanisms to minimize costs in situations that cannot or should not be avoided.

1.2.3 Physiological Mechanisms

Based on the connection of disgust to the insular cortex, which has also been identified as an important component of the Neuro-Immune-Axis in animals, Oaten and colleagues (2009) suggested that disgust elicits both behavioral and proactive physiological immune responses. Initial evidence for this theory was found in a study investigating interleukin 6 (IL-6) in blood (Schaller et al., 2010). Participants were shown either a 'disease slide show' depicting individuals with symptoms such as pox, skin lesions, and sneezing, or a 'gun slide show' displaying people with firearms. The study revealed that the stimulated production of IL-6 increased significantly after participants were confronted with the 'disease slide show' (Schaller et al., 2010). Two further studies demonstrated that exposure to disgust-inducing stimuli, such as images of rotten food, animals, and wounds, triggered an increase in various immune parameters. Participants showed elevated Tumor Necrosis Factor-alpha (TNF-α) levels and higher albumin concentrations in saliva than those exposed to control stimuli (Stevenson et al., 2012, 2011b). Another study found a significant increase in TNF-α, but not sIgA, in saliva after stimulation with disgusting odors (Juran et al., 2022). Brown et al. (2014) focused on variations in sIgA levels in saliva. Initially, they observed an increase in sIgA following exposure to disease-related stimuli (e.g., individuals with visible symptoms like sores, fever, and paleness) and mutilation-related stimuli (e.g., lacerations, burns, and amputations). However, they could not replicate these findings in a second study published within the same paper. Other studies also measured sIgA levels in response to disgust-evoking stimuli without a direct disease association, such as surgery videos and images of rotten food. Contrary to expectations, these studies reported a decrease in sIgA levels (Bosch et al., 2001; Stevenson et al., 2012, 2011b). Lastly, Stevenson et al. (2015) examined TNF-α, albumin, sIgA, and cortisol in saliva to determine whether disgust- and disease-related stimuli share the same proactive immune response. Contrary to previous studies, they found that neither the disgust images (dead animals, severe injuries, and rotten food) nor the disease images (hospital rooms, hospital staff, and

sneezing individuals) elicited a significant immune response compared to the negative control images (attacks, frightened/frightening people, disasters). In summary, evidence suggests a possible proactive physiological immune response; however, research in this area is contradictory and lacks consistency in study design, particularly regarding the definitions of disgust and disease video/images.

1.2.4 Interaction of Mechanisms

While Stevenson et al. (2015) were not able to find a proactive increase in the measured immune markers in the complete sample, a secondary analysis revealed that the participants who had a heightened sensitivity in core disgust showed an increase of TNF-α and sIgA after watching the disease and disgust images. This hints at a complementary interaction of the psychological mechanism – disgust- and the physiological mechanism – an increase of immune markers. Another study found support for the theory of a complementary interaction of the behavioral and physiological immune response, showing that avoidance behavior tends to increase after recent activation of the reactive PIS (Miller and Maner, 2011), while the authors interpreted this as a complementary interaction, it may also be evidence for a compensatory interaction as a recently activated reactive immune system could be evidence for a compromised physiological immune response infection (Arnold and Fuqua, 2020; Langford et al., 2020; LeVine et al., 2001; van der Sluijs et al., 2004). More clear evidence for a compensating relationship was found in men with a presumably more effective physiological immune response, showing reduced behavioral immune reactions (Kandrik et al., 2017), and women with a presumably less effective physiological immune response showing heightened behavioral immune reactions (Fleischman and Fessler, 2011). Higher germ aversion has also been linked to lower chronic basal inflammation (Gassen et al., 2018). Most of these findings are, however, correlative or secondary results.

1.3 Influences on the Mechanisms

1.3.1 Sex Steroid Hormones

Sex hormones are one important modulator of immune activation (Bouman et al., 2005; Grossman, 1984). This not only leads to sex differences in immunity, with females having a stronger immune response, producing stronger cellular and humoral immune reactions (Guerra-Silveira and Abad-Franch, 2013; Markle and Fish, 2014), which also may lead to increased autoimmunity in women (Klein and Flanagan, 2016). Further, it is also found as an intra-individual variation within the cycle of women, so do women seem to be more vulnerable to infection in the luteal phase (Pehlivanoglu et al., 2001; Wira and Fahey, 2008). Both testosterone and progesterone are known to downregulate the reactive immune response (Klein and Flanagan, 2016).

Regarding the proactive behavioral or cognitive immune response, research has found a sex difference in the behavioral mechanism disgust (Al-Shawaf et al., 2018). If disgust also varies depending on the menstrual cycle has been investigated, but results are mixed (for review: Stern and Shiramizu, 2022). However, there is a lack of research on whether endogenous sex hormones influence the proactive physiological immune system. The previous studies were either restricted to male participants (Stevenson et al., 2015, 2012, 2011b) or had an unbalanced sex ratio (Brown et al., 2014; Schaller et al., 2010). However, the influence of sex hormones on the reactive and proactive immune response may be one factor explaining (inter-) individual differences in the response and should hence be further investigated.

1.3.2 Context and Stimuli Specificity

The social and environmental context most likely also mediates the proactive immune response. Framing images within a disease context elicits stronger disgust responses than those presented in a neutral context (Santos et al., 2023). This heightened reaction aligns with enhanced information processing, such as increased eye movement. Similar effects have been observed

in other sensory modalities, including touch (Hunt et al., 2017) and olfaction (Chan et al., 2016), where individuals exhibit heightened sensitivity when associating a situation with disgust based on past experiences. It has been suggested that proactive immune responses are shaped by parental influence (Tybur et al., 2018) and previous experiences (Stevenson et al., 2011a). Through these experiences, individuals develop a perception of their vulnerability to disease (Duncan et al., 2009), which may, in turn, influence proactive immune mechanisms. For example, during the Coronavirus disease 2019 (COVID-19) pandemic, perceived vulnerability to disease (pVtD) was associated with increased preventative behaviors and reduced risk-taking (Stangier et al., 2022). The only study examining context-driven effects on a proactive physiological immune response was conducted by Brown et al. (2014). While they initially found an increase in sIgA following disease-related stimuli, their attempt to replicate the findings a few months later was unsuccessful. One proposed explanation is that the initial study took place during flu season, potentially enhancing participants' proactive immune responses due to a heightened disease context. If true, this suggests that the proactive immune system may adapt to specific contexts and even generate targeted responses. While previous research has focused solely on non-specific proactive physiological immune responses, it is possible that, under certain conditions, responses may be tailored to previously encountered pathogens. For instance, during the COVID-19 pandemic, visual cues of respiratory symptoms might have triggered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific immune responses in individuals who had been infected or vaccinated.

1.4 Brain activation related to proactive immune responses

While research has found first evidence for a proactive physiological immune response (Brown et al., 2014; Schaller et al., 2010; Stevenson et al., 2015, 2012) and the upregulation of salivary antibodies is possible without pathogen contact (Carpenter et al., 1998; Proctor and Carpenter, 2001), the mechanisms by which the Neuro-Immune-Axis detects sick individuals and triggers

a proactive immune response remain to be explored.

Regarding the behavioral immune response, research has found that the main brain region processing disgust is the insular cortex. Located in the Sylvian fissure under the frontal, temporal, and parietal opercula, the insular cortex is a structure with four to seven gyri and a large anterior as well as a small posterior lobule that are separated by the central sulcus (Naidich et al., 2004). It has previously been reported to play a key role in interoception (the perception of one's bodily states), top-down control of autonomic functions (i.e., heartbeat, gastric motility), and processing afferents from other brain regions (i.e., amygdala) (Gogolla, 2017). Disgusting photos (Wright et al., 2004), films (Harrison et al., 2010), imagining disgusting events (Jabbi et al., 2008), and recalling disgusting experiences (Fitzgerald et al., 2004) activate the anterior insula. Further, even processing of disgusted faces is associated with the anterior insula (Fusar-Poli et al., 2009; Jabbi et al., 2008; Phillips et al., 1997; von dem Hagen et al., 2009). Direct electrical stimulation of the insular cortex in monkeys provoked the typical disgust expression (wrinkled nose, lifting of the upper lip). It even made them discard their usually preferred food as something distasteful (Caruana et al., 2011). Further, during a disgust-conditioning paradigm, Klucken et al. (2012) found heightened activation in the insular cortex. A case study on a patient with lesions in the left insula found that the patient lacked the ability to recognize and experience disgust (Calder et al., 2000).

Historically, disgust has been ascribed to the insular cortex, while the amygdala has rather specifically been associated with the emotion of fear (Schäfer et al., 2005). However, the anterior insular cortex and the amygdala are closely connected (Augustine, 1985). The amygdala is an almond-shaped mass of grey matter inside the temporal lobe that comprises about 13 nuclei (Sah et al., 2003). Research now has found that confrontation with disgusting and repulsive stimuli not only activates the insular cortex but also the amygdala (Hayes et al., 2014; Kipps et al., 2007; Schäfer et al., 2005; Wicker et al., 2003). Most studies have used

disgusting images, making it challenging to draw implications on the proactive immune system regarding infection and sick conspecifics. Only two studies used images of individuals with subtle sickness/inflammation cues. While one study found activation in - among other regions - the posterior insular cortex (Regenbogen et al., 2017), the other could neither find an activation in the insula nor the amygdala (Leschak et al., 2022). Further, both studies let their participants rate the image's likeability during the presentation of stimuli, putting the focus away from infection aspects. There has been no imaging study presenting stimuli with apparent infectious symptoms. In animal studies, researchers found that inactivation of the posterior insular cortex leads to rats not differentiating between before-preferred healthy and sick individuals in their approach and avoidance behavior (Rieger et al., 2022). A study in mice showed that olfactory sickness cues of females led to an avoidance of mating behavior which was associated with a heightened activity in the cortical amygdala, this however seemed to be specific to the mating process as a similar activation could not be found after contact with unhealthy same-sex conspecifics (Kwon et al., 2021).

Neuroimmunology is a relatively new but heavily researched and important subject. It is now consent that the immune system and the nervous system do not act independently (Dantzer, 2018). One subject commonly used in this research is “sickness behavior”, which describes behaviors such as inactivity, sleepiness, reduced appetite, and hygiene when animals and humans need to conserve energy while fighting infections (Hart and Hart, 2019). The insula also seems to be an integral part of the manifestation of these sickness behaviors (Harrison et al., 2009b, 2009a; Lekander et al., 2016; Månsson et al., 2022). Further research has shown that the insular cortex plays a role in immune modulation (Hess et al., 2011), storing of immune-related information (Koren et al., 2021), and immune conditioning (Pacheco-López et al., 2005; Ramírez-Amaya et al., 1996; Ramírez-Amaya and Bermúdez-Rattoni, 1999) (for review of the insular cortex’s connection to the immune system see Rolls, 2023). In conclusion, studies on

the underlying mechanisms of brain activation in the context of visual sickness cues and proactive immune response may be important for a further understanding of the Neuro-Immune-Axis.

1.5 Present studies

The aim of this cumulative thesis was to establish a method that reliably measures a proactive physiological immune response and utilize this method to understand its underlying brain mechanisms, as well as to investigate aspects that might modulate and influence this response.

1.5.1 Measuring secretion changes of sIgA after disease and disgust stimulation

The first study [*2.1 Chapter I;* (Keller et al., 2022)] intended to establish a method that measures the proactive physiological immune response, while also accounting for the following shortcomings of previous studies: Firstly, most of the previous studies in this field tested a small number of participants: Schaller et al., 2010 tested 27 participants distributed across two groups, Stevenson et al. 2011 tested 92 participants in 3 groups, Stevenson et al. 2012 tested 68 participants in four groups, and Stevenson et al. 2015 tested 37 participants in a within subject design. Secondly, the used stimuli may have been inefficient to elicit proactive physiological responses, as some used stimuli only distantly related to disease and illness (such as hospital hallways) (Stevenson et al., 2015) or disease symptoms that may not be fought by the measured immune response, like skin lesions when measuring sIgA in saliva (Brown et al., 2014). In this study, we further moved away from images of stimuli and utilized five-minute videos that were put together out of short sequences and images. To disentangle what stimuli elicits a proactive physiological immune response we divided 107 participants into four groups watching either a control video (landscape), a concealed contagion video (people coughing and sneezing into tissues, lying in bed), an aerosol disease video (people coughing and sneezing openly, with aerosol flying) and a core disgust video (rotten food, dead animals, etc.).

We chose secretory IgA in saliva as immune parameter as it is one of the major players in the

first-line of defense against especially respiratory viruses with its functions of immune exclusion and intracellular neutralization (Strugnell and Wijburg, 2010) and can rapidly be upregulated (Carpenter et al., 1998; Proctor and Carpenter, 2001). Saliva samples were collected before and after stimulation and analyzed using the Atellica® NEPH 630 System, an automated nephelometric immunoassay system. In nephelometric immunoassays, antibodies (in this case, sIgA) are added to a solution with antigens. SIgA binds to the antigens and forms immunocomplexes that absorb and reflect light. The concentration of sIgA is then calculated based on a scatter of light passing through the photodetector (Töpfer, 2018).

Lastly, to measure behavioral immune responses, we utilized established questionnaires that capture trait and state disgust (Olatunji et al., 2007b) as well as perceived vulnerability to disease (Duncan et al., 2009). All testing was done remotely, and participants sent in their saliva samples via post, as data collection took place during strict COVID-19 restrictions.

We expected sIgA to increase after disease and disgust stimulation, with the aerosol disease video triggering the highest response, but not after the control, as an indicator of a proactive physiological immune response ((Brown et al., 2014; Schaller et al., 2010; Stevenson et al., 2015), *also see 1.2.3*). We further expected this increase to correlate with the behavioral immune measures ((Fleischman and Fessler, 2011; Stevenson et al., 2015), *also see 1.2.4*).

1.5.2 Measuring response of disease-specific sIgA

Previous studies mainly focused on a general proactive physiological immune response with no specificities. In our first study [*2.1 Chapter I;* (Keller et al., 2022)] findings suggested that specific videos elicit different responses in total sIgA, therefore and based on the theory that the proactive immune responses may be influenced by context (*see 1.3.2*) we conducted our second study [*2.2 Chapter II*, (Keller et al., 2023)]. As our research took place during the mid to end of the COVID-19 pandemic, and most individuals had formed antibodies against the Sars-CoV-2 virus by infection or vaccination, we focused on these specific sIgAs. Here, we

adapted the general procedure described in *Chapter I*, but with slight modifications: Firstly, we combined the aerosol disease and concealed contagion videos into one five-minute video. Secondly, we switched to a within-subject design with participants watching either the disease or the control video on two consecutive days. Thirdly, participants were not tested remotely, but in our in-house testing lab. Fourthly, unlike the previous study, we used a MULTICOV-AB, a multiplex Sars-CoV-2 immunoassay (Becker et al., 2021a, 2021b) to determine Sars-CoV-2-specific sIgA levels. This immunoassay analysis 20 antigens simultaneously, 6 of which are specific to Sars-CoV-2 (Spike Protein, receptor binding domain (RBD), S1 & S2 domain, nucleocapsid, and nucleocapsid N-terminal domain) (Becker et al., 2021a). The assay is a bead-based multiplex assay, where all antigens are covalently bound and therefore immobilized to paramagnetic beads, which have red and infrared-fluorophore color codes. The immobilized antigens then bind to the specific sIgA domains and are detected by goat-anti-human IgA; any unbound antigens were washed away. Readouts were based on luminescence of magnetic beads in a Luminex FLEXMAP 3D instrument (Becker et al., 2021b).

Based on the findings of *Chapter I* and the hypothesis of context driving proactive immune responses (Brown et al., 2014; Santos et al., 2023; Stangier et al., 2022), we expected the SARS-CoV-2-specific sIgA secretion to increase after the disease video, displaying people with respiratory symptoms, but not after the control video. We further expected this increase to correlate with our behavioral questionnaires (trait & state disgust, pVtD, and interoceptive feelings (adapted from Kupfer et al., 2021).

1.5.3 Measuring the influence of the menstrual cycle on sIgA response

Sex hormones modulate many reactive and behavioral immune responses (*see 1.3.1*). Therefore, assuming they also modulate the proactive immune response would be reasonable. In order to investigate this, we tested women in different cycle phases and further compared them to women taking oral contraceptives in our third study [*2.3 Chapter III;* (Keller and

Diekhof, 2024)]. In a similar remote, between-subject study design as in Chapter I, we showed female participants a video that combined the concealed contagion and aerosol disease videos. We assessed three groups of women (two groups of women with a natural menstrual cycle, being either in the luteal phase or the follicular phase, and one group of women in the active taking phase of hormonal contraceptives). Additionally, to the sIgA saliva samples (which again were analyzed as in *Chapter I*), the participants gave three saliva samples in the morning, which we analyzed for the female sex hormones progesterone and estradiol utilizing enzyme-linked immunosorbent assays (ELISA). Similarly to the previously described immunoassays, the sex hormones in saliva bind to antigens, which are then detected by antibodies; all non-detected antigens are washed out (Clark et al., 1986). Readouts are made by a spectrophotometer measuring the absorbance of light.

We hypothesized that the immunosuppressive effect of elevated progesterone ((Bouman et al., 2005; Klein and Flanagan, 2016; Pehlivanoglu et al., 2001; Wira and Fahey, 2008), *also see 1.3.1),* during the luteal phase would reduce or eliminate the proactive sIgA response to the disease-primer video. We further expected differences in our behavioral measures (state and trait disgust, pVtD) between the three groups.

1.5.4 Measuring underlying brain mechanisms of proactive immune responses with functional magnetic resonance imaging (fMRI)

In order to understand how our visual respiratory disease symptoms are processed in our brain and then further lead to the production of sIgA in saliva, we conducted our fourth Study [*2.4 Chapter IV;* (Keller and Diekhof, 2025)]. In this study, we adapted our method into a paradigm fit for functional magnetic resonance imaging (fMRI). Magnetic resonance imaging (MRI) is a non-invasive method that enables the creation of sectional images of body parts. This method is based on the spin angular momentum of hydrogen nuclei, which is the outcome of the rotational motion of a hydrogen nucleus around its axis. The spin of hydrogen nuclei is always

present and is randomly oriented. When placed in a strong external magnetic field, the hydrogen nuclei align either parallel (low-energy state) or antiparallel (high-energy state) to the field. To capture an image, radio frequency pulses are used to excite the hydrogen nuclei, temporarily flipping them into a higher energy state. MRI measures the time it takes for longitudinal magnetization to recover (T1). The length depends on the magnet's size and the molecular structures around the nucleus (e.g., grey matter has a T1 of 900 ms, fat one of 250 ms) (Landini et al., 2018). The MRI also measures the time constants associated with loss of transverse magnetization due to interactions with their environment (T2: spin-spin interaction & T2*: T2 plus magnetic field inhomogeneities), which is an important timing for functional MRI, needed for measurements of brain activity. Neural activity increases local blood flow (Matthews and Jezzard, 2004). Due to diffusion-limited oxygen uptake, more oxygenated blood is supplied to active brain regions than the neurons actually use. This results in a local excess of oxygenated hemoglobin, which is essential for and increases the strength of the BOLD (blood oxygenation level dependent) signal utilized in fMRI (Logothetis, 2008). The BOLD signal is based on the fact that oxygenated hemoglobin is diamagnetic and deoxygenated hemoglobin is paramagnetic. Oxygenated and deoxygenated hemoglobin produce different magnetic properties, affecting T2*. Deoxygenated hemoglobin introduces local magnetic field inhomogeneities, accelerating T2* decay. A constant measuring of the magnetic T2* signal can therefore measure the blood flow in specific brain regions (Matthews and Jezzard, 2004; Ogawa et al., 1990). The BOLD signal can be converted into brain activity utilizing specific software (in our case, SPM 12) that corrects, processes, and analyzes the fMRI data (Flandin and Novak, 2020).

In our final study [*Chapter IV*], participants watched short clips of either sneezing people, neutral people, or matched backgrounds without people. We captured saliva before, during, and after the stimulation in the fMRI scanner, which was analyzed as in Chapter I. Further, participants rated each sequence regarding the disgust potential and perceived infectability on

a 6-point Likert scale.

Firstly, we expected sIgA to increase throughout the experiment, due to stimulation with disease-related stimuli amongst the neutral ones, as previously seen in *Chapters I, II, and III*. As the insula and the amygdala have been associated with disgust and may be part of the Neuro-Immune-Axis ((Harrison et al., 2009b; Hess et al., 2011; Rolls, 2023), *also see 1.4*), we expected heightened activity in these brain regions when watching sequences of sick people compared to the two control conditions. We further expected an interaction between the ratings (disgust and infectability), an increase of sIgA (based on the previous *Chapters I, II, and III*), and the activation in the regions associated with the Neuro-Immune-Axis.

2. Publications

2.1 Chapter I: Disease-related Disgust promotes antibody release in human saliva

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Disease-related disgust promotes antibody release in human saliva

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ABSTRACT

The behavioral immune system (BIS) comprises manifold mechanisms, that may assist the physiological immune system (PIS) in counteracting infection and can even reduce the risk of contagion. Previous studies have found initial evidence for possible interactions between the two systems. However, most of these findings were correlative and have not been replicated. Further, none of these studies examined whether disease stimuli that indicate an enhanced airborne transmission risk may trigger a different immune response in comparison to stimuli that predominantly evoke core disgust. In the present study, we employed a video-priming approach to get further insight in the influence of the perception of disgust- and disease-related stimuli on the rapid physiological immune response, as indicated by changes of secretory immunoglobulin A (S-IgA) in saliva. We created three video primers that represented different categories of disgust- and/or disease-associated content. Two of the videos showed disease-related situations that were associated with contagious respiratory virus infections, varying in concealment of aerosols. The third video incurred no heightened airborne contagion risk, but comprised situations that are known to elicit core disgust, such as rotten foods, decaying animal carcasses, or cockroaches. A fourth video acted as control showing landscape impressions. The different video primers varied in their contagion risk and disgust-evoking potential. Given the role of S-IgA in the mucosal immune defense, we expected differences in the S-IgA response between the two videos indicating a heightened airborne contagion risk and the core disgust video, with the highest S-IgA to occur after the aerosol video. For this, we used the data of 107 healthy participants in a between-subjects design with the four video primers. We found a significant increase of S-IgA in response to both the disease- and the disgust-related videos, which correlated positively with the perceived contagion risk of the displayed situations. Nevertheless, there was no significant difference in the increase between the three disease- and disgust-related videos. We also found that people with a high contamination disgust produced less S-IgA in such situations, which is a hint for a compensating relationship between the BIS and PIS. Our observations suggest that the mere visual perception of videos showing realistic situations of an increased contagion risk can elicit a heightened release of salivary antibodies.

1. Introduction

The physiological immune system (PIS) has evolved due to the constant pathogen threat in the environment. While both the unspecific, innate and the specific, adaptive immune system are highly effective, most functions of the PIS are very resource consuming and can have negative consequences when misdirected (McDade, 2003). This led scientists to propose the theory of a behavioral immune system (BIS), first described as such by (Schaller and Duncan, 2007). The BIS comprises mechanisms that aim to proactively avoid pathogens even before such pathogens are coming in contact with the organism. Thus, activation of the BIS might reduce the necessity to activate the PIS.

As a complex system, the BIS may detect potential pathogens, and trigger defensive responses like disgust, avoidance behavior, social exclusion and sickness behavior (Schaller and Park, 2011). The ongoing Covid-19 pandemic has shown that particularly social avoidance can be very effective in reducing contagion risk. Yet, the various mechanisms of the BIS may also incur costs for the individual. While social distancing has proven as an effective method to reduce the spread of Sars-CoV-2 (Qian and Jiang, 2020), it has led to economical (Tuzovic and

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Kabadayi, 2021), social and psychological distress in many individuals (Marroquín et al., 2020). Even on the small scale, pathogen avoidance carries costs of lost opportunity (e.g., the reduction of social contacts can lead to lower mating chances), hence researchers have suggested that the BIS and PIS are interconnected to optimize the cost/benefit ratio of both (Gangestad and Grebe, 2014; Oaten et al., 2009; Schaller and Park, 2011). As the BIS is mainly triggered by sensory cues (e.g.: visual stimuli and auditory), the interaction between the two systems would most likely be part of the neuro-immune-axis, acting either over endocrine mechanisms or the autonomic nervous system (for review Wrona, 2006). The exact route by which the brain and the immune system interact in this context has not been investigated. Nevertheless, to do so it is important to find a method to reliably trigger the BIS-PIS interaction, before moving experiments into neuro-pathway fields (e.g., functional neuroimaging).

Previous studies found initial evidence for possible interactions between the BIS and the PIS: (1.) avoidance behavior increased after recent activation of the PIS (Miller and Maner, 2011), (2.) behavioral immune responses were lower in men with a proposedly more effective physiological immune response (Kandrik et al., 2017), and (3.) higher germ aversion predicted lower chronic basal inflammation (Gassen et al., 2018). However, most of these findings were correlative and findings have not been replicated (Tybur et al., 2020).

A more direct measure to investigate the relationship between the PIS and BIS is to experimentally confront participants with disgust evoking stimuli, in order to provoke behavioral and physiological immune responses. Disgust may have evolved as a response to objects that represent a potential threat of (infectious) diseases (Curtis et al., 2004; Tybur et al., 2009). The emotion of disgust also correlates with avoidance behavior (Campbell et al., 2020; Dorfan and Woody, 2011). Two studies from the group of Stevenson found an increase in various immune parameters after presentation of disgust evoking stimuli, such as pictures of rotten food, animals and wounds. These included a rise in body temperature, Tumor Necrosis Factor alpha (TNF-a) and albumin levels relative to control stimuli (Stevenson et al., 2011, 2012). In another study, Schaller et al. (2010) assessed the relationship between the BIS and the PIS from a somewhat different methodological angle. They used pictures of people showing disease symptoms. These included morphological (pox, skin lesions) and behavioral (sneezing, coughing) characteristics of various illnesses. While participants were not more disgusted by the disease-related than the control pictures, the study nevertheless found an increase in the interleukin-6 (IL-6) blood concentration after the disease stimuli.

Setting the focus on the mucosal immune response Brown et al. (2014) measured the change of secretory immunoglobulin A (S-IgA) in saliva. While they initially found an increase in S-IgA after showing disease (people infected with diseases, showing symptoms like sores, fever, paleness) and mutilation stimuli (lacerations, burns and amputations), they failed to replicate their own results in a second study, which was published in the same paper. S-IgA has also been measured in studies that presented disgust-evoking stimuli, with no direct disease-association (surgery video, rotten food, etc.). However, contrary to expectation, S-IgA rather seemed to decrease in these studies (Bosch et al., 2001; Stevenson et al., 2011, 2012). In an attempt to disentangle if disgust- and disease-related stimuli rely on the same pathways, Stevenson et al. (2015) therefore compared the immune responses after disease- and disgust-related stimuli. For this purpose, they created two sets of images, of which one was classified as disgusting, but not directly disease-related (e.g., picture of garbage, dead animals, mutilated body parts), while the other was intended to evoke disease-related concerns without being overly disgusting (e.g., pictures of hospital ward, x-rays, sneezing). Both sets of images failed to increase S-IgA and other salivary immune parameters (TNF-α, albumin, cortisol), part of which were previously identified to be disgust-sensitive (Stevenson et al., 2011). Only after a secondary analysis, Stevenson et al. (2015) found an increase in S-IgA and TNF- α in a subgroup of people with higher-than-average self-reported disgust sensitivity.

In the present study, we took a similar approach as Stevenson et al. (2015) to get further insight in the effect of disgust- and disease-related stimuli on the physiological immune response. One may argue that the nature of the immune response probably depends on the class of pathogens presented (Bradshaw and Gassen, 2021). Therefore, we decided to use disease-associated stimuli, which were more realistic and were also more specifically associated with respiratory contagious pathogens than the stimuli used in previous studies. In contrast to our stimuli, the stimuli of previous studies, comprised images of x-rays or hospital hallways (Stevenson et al., 2015) or of sick people, who mostly exhibited no sign of respiratory illnesses (e.g., people with skin rashes or open wounds) (Brown et al., 2014; Schaller et al., 2010). For this purpose, and in contrast to previous studies, our stimulus material also included short videos of people with visible signs of sickness or of disgust-evoking scenes. Further, we created three different stimulus sets that each represented a different level of contagion risk and varied in their disgust-evoking potential. These included two disease-related sets of people displaying obvious symptoms of respiratory diseases (e.g., the flu, a common cold) that either directly or indirectly implied an increased airborne contagion potential, i.e., the aerosol and the concealed contagion category. The aerosol category thereby included videos and pictures of openly sneezing people, most of them oriented towards the camera, with more or less visible aerosol spread like flying droplets or sputum. Conversely, in the concealed contagion category we showed people with flu-like symptoms (e.g., videos and pictures of people looking pale and lying in bed, obviously suffering from headache and fever, or having a red nose). Some of them were also sneezing or coughing, but they were concealing it by covering their nose or mouth. The third stimulus set focused on sickening disgust-evoking stimuli (core disgust category; e.g., videos and pictures of parasites, rotten foods, dead animals) that would need direct body-contact (e.g., by being touched or actively ingested) to induce an illness. Finally, we also included a control video without an association to disgusting or disease-evoking stimuli, which consisted of landscape videos (e.g., city panoramas, aerial views of busy crossroads).

Since the pathogens associated with respiratory diseases enter the body through mucosal tissues of the respiratory tract, we focused on the mucosal immune system of the oral cavity, specifically the change of salivary S-IgA. S-IgA is the main mucosal immunoglobulin and plays an important role in the first-line-immune-defense against pathogens that enter the body through mouth or nose (Woof and Mestecky, 2015). It is constantly secreted into saliva at a base rate, and can be rapidly upregulated by (para-)sympathical (Carpenter et al., 1998) and mechanical stimulation (Proctor and Carpenter, 2001). S-IgA is part of several immunological processes such as immune exclusion, i.e., the binding of antigens and prevention of attachment to epithelia cells, and intracellular neutralization, i.e. the neutralization of viral replication in epithelial cells, which play an important role in infection immunity (Corthésy, 2013; Strugnell and Wijburg, 2010). The responsiveness of the S-IgA secretion to visual stimulation by disease- and disgust-related stimuli has been assessed previously (Bosch et al., 2001; Stevenson et al., 2011, 2012, 2015; Brown et al., 2014), but results have been inconsistent.

In our study, young healthy participants watched one of the four videos and answered an online survey to assess inter-individual state and trait differences in the BIS (see details below). Saliva samples were collected at baseline and twice after the video, from which changes in S-IgA were assessed. Given the well-established role of S-IgA in the mucosal immune defense (Woof and Mestecky, 2015), we expected that the participants in the aerosol video group would experience the spreading aerosol directed towards them as a higher threat of contagion, compared to the concealed contagion disease stimuli and also to the core disgust stimuli. Consequently, we expected the highest S-IgA response in the aerosol group compared to the concealed contagion group and the other two groups (i.e., core disgust and control group). Based on

previous studies, investigating the S-IgA response to disgust evoking stimuli (Bosch et al., 2001; Stevenson et al., 2011, 2012), we expected the secretion of S-IgA to go down in the group of participants, who watched the core disgust primer. This would also be consistent with the assumption that the mere visual perception of parasites or rotten food is not expected to trigger a mucosal immune response in the oral cavity (Bradshaw and Gassen, 2021).

To shine more light on inter-individual differences in the behavioral immune system we also utilized established questionnaires that capture trait as well as state disgust (Disgust Scale-Revised (Olatunji et al., 2007),) and self-reported disease vulnerability (pVtD, (Duncan et al., 2009). We expected changes in S-IgA depending on these traits in interaction with the category of the video primer. Based on the finding that S-IgA increases in individuals with higher than average trait disgust (Stevenson et al., 2015), we expected that trait disgust may be associated with a stronger increase in S-IgA, especially after the core disgust primer. Previous studies showed that people with a high self-reported vulnerability to disease show a lower physiological immune response (reduced inflammatory markers in blood, decreased general fitness of the immune system) (Gassen et al., 2018; Kandrik et al., 2017). This supports the theory that the BIS may have evolved to relieve the PIS. Based on this, we expected that participants with a higher pVtD score should exhibit a lower response in S-IgA secretion, and particularly so with regard to the videos with disease-related content.

2. Materials & methods

2.1. Participants

In our pre-registered https://osf.io/9hxpt/) between-subjects design we confronted the participants with three different disease- and/or disgust-related stimulus sets. In addition to that, we also included a control group, who watched a video with neutral content (landscape impressions and city panoramas). A power analysis with G*power (Faul et al., 2007) indicated that a sample size of 96 participants (24 individuals in each group) would provide sufficient power (1-β = 0.90) to detect a large effect of f = 0.40 with α = 0.05. To compensate for dropouts we tested five additional subjects per condition. We recruited 116 participants (47 m/69 f) on the university campus, through online advertisements and via social media. We only invited healthy individuals to participate, who (a) indicated German as a native language, (b) were of legal age but not older than 35 years, (c) were not smoking regularly, (d) had no hormonal, genetical, or other chronical diseases, (e) had not been vaccinated in the last 3 weeks, and (f) were willing to participate online for the approximate duration of 1 h. Female participants were only included, if they used hormonal contraception. Data collection took place between May and October of 2021. Participants received a financial reward of 12 Euros for completing the appointment. We obtained informed consent from all participants and the procedure was approved by the local ethics committee *"Ethikkommission der Ärztekammer Hamburg"* and conformed with the Declaration of Helsinki.

2.2. Stimuli

The participants were randomly assigned to one of four videos (i.e., video primers). These videos differed in their disgust-evoking potential and their disease-related content. The aim was to compare three disgust- and/or disease-associated primers, which triggered different degrees of disgust and fear of contagion. All videos showed a combination of short video clips and pictures that were assembled in a video of 1:20 min length. In order to achieve a sufficient priming effect, each video was repeated once (total video length = 2:40 min). The videos consisted of royalty free material from pages like pexels.com and pixaby.com, while some were bought of istockphoto.com. The stills in the Core Disgust video were taken from the DIRTI-Database (Haberkamp et al., 2017). Most of the openly sneezing people were from the *"Bless-you"* video, by Ulf Lundin. For a detailed description, see Supplementary Tables 1–4.

Aerosol Disease Video (A): This video was intended to trigger a high level of disgust and high fear of contagion. It comprised video clips and pictures of people, who were sneezing unconcealed, either directly into the camera or in the vicinity, whereby some visibly emitted aerosols.

Concealed Contagion Disease Video (CC): This video showed people sneezing without emitting aerosols (e.g., sneezing into a tissue). Other people in the video showed visible signs of sickness, such as looking feverishly or laying sick in bed. We predicted this video to elicit less disgust and a medium to high fear of contagion, compared to the Aerosol Disease Video.

Core Disgust Video (CD): Presuming that non-airborne disease threats trigger a different immune response, we created a video showing rotten food, dead animals with maggots, rats, and other disgusting items. Through this video, we tried to elicit a similar disgust response as in the Aerosol Disease Video, yet with a significantly lowered fear of contagion.

Control Video (C): In this video, we combined video clips and photos of buildings, skylines, traffic intersections and other landscape views. This video was intended to trigger no disgust- or disease-related responses.

2.3. Online surveys

Our participants underwent online surveys (see 2.5 Procedure below) that were programmed with the software LimeSurvey and Inquisit 6 (Millisecond, 2021). The Zoom application was further used for interactions between the participant and the experimenter in the breaks between the different tasks. Throughout the experiment, they evaluated their trait disgust, vulnerability to disease and changes of state disgust, mood, etc. related to the video in the following Questionnaires.

2.3.1. Before the video

Mood Scale: Participants rated how they feel, answering 24 questions adapted from the German MDBF (Mehrdimensionaler Befindlichkeitsfragebogen) mood-scale from 1 to 5 (1 = not at all; 5 = a lot). For example, tired, satisfied, happy, nervous, etc. (Steyer et al., 1997).

Disgust Scale-Revised (DS-R): Participants evaluated their trait disgust sensitivity on the modified version of the Disgust Scale established by Haidt et al. (1994) and revised by Olatunji et al. (2007). This scale consisted of 17 items, eight of these were true-false items with statements like "*I might be willing to try eating monkey meat, under some circumstances.*", for which participants indicated their agreement on a 5-point Likert-scale (from 0 = '*Strongly disagree*' to 4 = '*Strongly agree*'). The rest of the items had the participant rate situations from 0 = '*Not disgusting at all*' to 4 = '*Extremely disgusting*', for example, "*While you are walking through a tunnel under a railroad track, you smell urine.*". The resulting score were then categorized into the Core-Disgust-Score (12 items), and the Contamination-Disgust-Score (5 items).

Perceived Vulnerability to Disease (pVtD): Participants evaluated their perceived Vulnerability to Disease, by using a 15-item self-report instrument designed by Duncan et al. (2009). These 15 items included statements like "*In general, I am very susceptible to colds, flu and other infectious diseases.*" and "*I prefer to wash my hands pretty soon after shaking someone's hand.*". The participants had to evaluate each item with a 7-point Likert-scale (1 = '*Strongly disagree*' to 7 = '*Strongly agree*'). The resulting score was further categorized into the sub-scores Germ-Aversion (8 items) and the Perceived-Infectability- (7 items).

2.3.2. After the video

Video-Questionnaire: Participants had to answer questions about the video content. We first asked three questions that required a recall of details, such as "*How many elderly men were portrayed in the video?*". The participants had a choice between five options, such as "*None*", or "*Only 1 elderly man*". Furthermore, participants were shown 15 pictures of which 10 were screenshots from the video previously shown, while 5

were not shown in the video. The participants were asked, "*Was this person/situation portrayed in the video?*" and had to choose between the options "*Yes*" and "*No*". These questions allowed us to implicitly evaluate whether participants had payed attention to the details of the video, participants that answered less than 50% correct would be excluded.

Lastly participants answered explicit questions on how much attention they had paid during the video and how realistic they would rate them. For this we used nine statements, such as "*I was distracted during the video*." and "*If this was a real situation I would have become sick.*" (Contagion risk question), which they answered on a 5-point Likert scale form −2 '*Completely incorrect*' to +2 '*Completely correct*'. For analysis this scale was converted to a span from 0 to 4.

Modified Differential Emotions Scale (mDES): Participants were further asked to recall the explicit feelings they experienced while watching the video. For this "absolute recall task", we asked them how they felt, while watching the video using 6 statements, such as "*How strong was your feeling of disgust, antipathy and revulsion while watching the video?*". The statements about feelings had to be rated on a 9-point Likert-scale from 0 "not at all" to 8 "completely"(Brandenburg and Backhaus, 2015; Fredrickson, 2013).

Relative-Feelings: Participants were asked for the relative change in their feelings compared to their emotional state before the video, using another 6 statements, such as "*After the video I feel weaker and sickly.*", which they answered on a 5-point Likert-scale from −2 *'I feel much less like that than before the video*' to +2 *'I feel a lot more like that than before the video*'.

Both the absolute (mDES) and the relative recall of feelings depicted an identical number of negative (e.g., stress, disgust) and positive feelings (e.g., amusement, inspiration) and reflected emotional state after the video.

Evaluation of Stimuli: Participants evaluated the amount of disgust in relation to the scenes and picture in the videos. For this, we used 37 screenshots from all four videos independent of whether they had been shown to the respective participant (each participant watched only one of the videos, which was randomly assigned). The participants had to decide on how they felt, when watching the situation or person on a given screenshot. For this, they used a 7-point Likert-scale from 1 "*no disgust at all*" to 7 "*extremely strong disgust*". Through these questions, we were able to evaluate the actual impact of the stimuli in terms of their disgust potential, and were also able to assess the influence of the video prime on subsequent stimulus evaluation. For results of every single stimuli, see Supplementary Tables 1–4.

2.4. Saliva samples

The participants received a kit for saliva collection at home. It was sent by mail, since the restrictions related to the COVID-19-pandemic precluded tests in our computer lab at the university. The kit included three 2 ml Eppendorf Tubes and an instruction of proper saliva sample collection. Once the experiment was completed, the envelope with the samples was immediately sent back to the institute. During the test session, the participants were asked to take their saliva samples at predefined time points during the surveys. The experimenter, who was blind to the participant's condition, instructed and monitored the saliva sample collection via Zoom calls. The experimenter also answered any study-related questions prior and after the test session via Zoom. However, the subject was left in private while he/she watched the video prime and filled in the online-survey (questionnaires and demographic data). Upon arrival at the institute, the saliva samples were frozen at −20 °C. For analysis, the frozen saliva samples were sent on dry ice to the MVZ Laboratory Volkmann, Karlsruhe, Germany. There, an immunonephelometric analysis to determine the concentration of S-IgA in saliva was performed on the Atellica® NEPH 630 System S-IgA.

2.5. Procedure

The computer test was conducted in the afternoon (between 1pm and 5pm). Previous evidence suggests that S-IgA shows its daily peak in the morning and then drops to a stable level in the afternoon (Shirakawa et al., 2004). In the beginning of the test session, participants were informed about the general purpose of the study, the opportunity to abort data collection at any time, as well as aspects concerning anonymity and safety. Then, participants provided demographic data on aspects such as age, gender, and current state of health. They also reported, whether they had been exposed to any stressors, such as smoking, sports, alcohol within the last 48 h, as well as current and previous diseases, before moving on to the Mood Scale. After that, participants collected the first saliva sample (baseline sample). The experimenter documented the time participants needed to collect 1.5 mL of saliva. The duration of sample collection was also documented for the next two samples. On average, participants needed $\bar{x} = 3.88$ min/sample. Afterwards the participants moved on to answer the DS-R and pVtD. After completion, the participant was linked to the online software Inquisit. There, each participant was randomly assigned to one of the four video primers. The second saliva sample was taken immediately after the end of the video primer. It was followed by the Attention, the mDES and the Relative-Feelings questionnaires. After this last survey ($\bar{x}_{duration} = 5.5$ min), participants were asked to give the third and last saliva sample. Then, participants completed the evaluation of the stimuli (also see Fig. 2).

2.6. Data analysis

For data analysis, we calculated S-IgA concentration against the time it took the subjects to fill the tube with 1.5 ml of saliva [(mg/dl)/min]. All data was tested for deviation from a normal distribution using the Kolmogorov-Smirnov test with a statistical threshold of $p = .050$. Since all tests were significant, we used non-parametric post-hoc tests.

Firstly, we used Kruskal-Wallis-tests, with Primer as independent and post-video evaluation scores as dependent variables to assess how primer category affected the evaluation of the video content. Again, we utilized Mann-Whitney-U tests as post-hocs.

Secondly, we assessed whether the increase in S-IgA was affected by the category of the video primers. For this, we utilized a general linear model for repeated measures (GLM) with Sample (Baseline, Sample 2 and Sample 3) as within-subject factor and Primer (Aerosol, Concealed Contagion, Core Disgust and Control) as between-subjects factor. We used the Mann-Whitney-U test as post-hoc test. All post-hoc analyses included Bonferroni adjusted p-values (p_a).

For the assessment of correlations between the video-evoked increase in S-IgA and (1.) state disgust, (2.) trait disgust, (3.) trait VtD, and (4.) and fear of contagion, we employed the spearman rank correlation. All statistical analyses were performed in IBM SPSS Statistics 27. Figures were created with R Studio and MS-Excel.

3. Results

We excluded data of 9 participants, who were exposed to more than three of the stressors listed in the initial survey in the 48 h before the test. The final analysis was therefore based on data of 107 participants, with an average age of 24.72 years ($\sigma = 3.60$ years). Of these, 27 participants received the Concealed Contagion Disease Video (16 f/11 m), another 27 watched the Core Disgust Video (18 f/9 m) or the Control Video (16f/11 m), and the remaining 26 participants received the Aerosol Disease Video (14f/12m).

3.1. Evaluation of the video primers

In order to test, whether participants indeed differentially perceived the three videos in terms of their disgustingness and contagion risk we

analyzed two survey questions, which were asked after the video. The first question was part of the mDES and asked: "*How strong was your feeling of disgust, antipathy and revulsion while watching the video?*". The Kruskal-Wallis-Test showed that the four video primers differed significantly in disgustingness ($H = 56.63$; $df = 106$; $p < .001$, $\eta^2_H = 0.492$, see Fig. 3a). While the Aerosol Disease Video and the Core Disgust Video elicited an equal amount of disgust (A vs. CD: $U = -5.98$, $p = .477$, $p_a = 1$, $\eta = 0.010$), the Concealed Contagion Disease Video elicited less disgust than these two (CC vs. A: $U = 23.92$, $p = .004$, $p_a = .025$, $\eta = 0.197$; CC vs. CD: $U = -29.90$, $p < .001$, $p_a = .002$, $\eta = 0.332$). Furthermore, all three disgust- or disease-related primers triggered higher disgust ratings than the Control Video (C vs. A: $U = 50.09$, $p < .001$, $p_a < .001$, $\eta = 0.626$; C vs. CC: $U = 26.17$, $p = .002$, $p_a = .009$, $\eta = 0.353$; C vs. CD: $U = 56.07$, $p < .001$, $p_a < .001$, $\eta = 0.721$).

Regarding the contagion risk associated with the stimuli in the video, we asked participants to rate the following statement in the Video-Questionnaire: "*If this was a real situation I would have become sick.*". The Kruskal-Wallis-Test showed that the four primers differed significantly in the perceived contagion risk ($H = 25.39$; $df = 106$; $p < .001$, $\eta^2_H = 0.217$, see Fig. 3b). We found that all three disgust- or disease-related primers triggered a higher fear of contagion compared to the Control video (C vs. A: $U = 33.10$, $p < .001$, $p_a < .001$, $\eta = 0.292$; C vs. CC: $U = 36.80$, $p < .001$, $p_a < .001$, $\eta = 0.439$; C vs. CD: $U = 25.93$, $p = .001$, $p_a = .009$, $\eta = 0.231$). However, there was no significant difference in the rating between the three primers themselves (CD vs. A: $U = 7.17$, $p = .382$, $p_a = 1$, $\eta = 0.016$; CD vs. CC: $U = 10.868$, $p = .181$, $p_a = 1$, $\eta = 0.036$; A vs. CC: $U = -3.694$, $p = .650$, $p_a = 1$, $\eta < 0.001$).

4. Primer-induced changes in S-IgA

Using a 4 (Primer) x 2 (Sample) GLM, we found a significant main effect of Sample ($F(1, 103) = 15.75$, $p < .001$, $\eta^2_p = 0.133$) as well as an interaction of Sample and Primer ($F(3,103) = 3.09$, $p = .030$, $\eta^2_p = 0.083$) on S-IgA. To further investigate these effects, we split the data by Primer and performed Wilcoxon signed-rank tests between the Baseline sample and Sample 2, which was collected directly after the video. We found that watching any of the three disgust- and/or disease-related primers led to a significant increase of S-IgA after the video, while the control primer did not (see Table 1, Fig. 4).

To further compare these increases between video primers, we calculated the difference between Sample 2 and the Baseline sample (ΔS-IgA). Yet, we found that none of the four primers showed a significantly larger increase than any of the others after the Bonferroni correction. (A vs. CC: $U = 341.0$, $p = .859$, $p_a = 1$, $\eta < 0.001$; A vs. CD: $U = 317,5$,$p = .551$, $p_a = 1$, $\eta = 0.007$; A vs. C: $U = 256,0$, $p = .091$, $p_a = .546$, $\eta = 0.054$; CC vs. C: $U = 239.0$, $p = .030$, $p_a = .180$, $\eta = 0.087$; CC vs. CD: $U = 313.0$, $p = .373$, $p_a = 1$, $\eta = 0.015$; CD vs. C: $U = 279.5$, $p = .141$, $p_a = .846$, $\eta = 0.040$; also see Supplement Fig. 1).

4.1. Influence of state disgust and perceived contagion risk on S-IgA

We correlated the rating of how disgust evoking presented stimuli were from (1.) the Evaluation of Stimuli, and (2.) the mDES ("*How strong was your feeling of disgust, antipathy and revulsion while watching the video?*") with the ΔS-IgA. This was done to investigate if state disgust, evoked by the presented videos, was related to the increase in S-IgA concentration. We found no significant correlations between the two state disgust measures and the ΔS-IgA (Video evaluation: $r_s = 0.040$, $p = .683$; mDES question: $r_s = 0.045$, $p = .644$).Furthermore, we correlated the above-mentioned rating of the perceived contagion risk with the increase of S-IgA. Here, we found a significant correlation ($r_s = 0.230$, $p = .018$), which is displayed in Fig. 5.

Fig. 1. Examples from the four stimulus sets: a) Aerosol-Primer (© Ulf Lundin); b) Concealed Contagion-Primer (Mojep (pixaby.com)); c) Core Disgust-Primer (Haberkamp et al., 2017); d) Control-Primer (Ricardo Esquivel (pexels.com)).

In fact, only a subgroup of the participants, who watched the two disease-related primers perceived the video content as a realistic contagion risk, hinting that the video material might not have been realistic enough for each observer. This finding led us to conduct a secondary analysis, in which we excluded participants from the analysis, who did not rate the disease video primers as a potential contagion risk ($n = 25$). In addition, we also excluded participants, who in turn perceived the control primer as a contagion risk ($n = 3$). After excluding these 28 participants, we combined the two disease-related primers (Aerosol and Concealed Contagion) to one Disease Primer ($n = 28$), in order to keep the sample size comparable to that of the other two primer groups. The correlation between contagion risk and ΔS-IgA stayed significant after exclusion of these cases ($r_s = .255$, $p = .023$). Running the same GLM as before, we still found the significant main effect of Sample ($F(2, 76) = 11.32$, $p = .001$, $\eta^2_p = 0.130$) and the interaction between Sample and Primer ($F(1,76) = 6.64$, $p = .002$, $\eta^2_p = 0.149$, Fig. 6).

Using the same post-hoc analysis we found a significant increase in the disease- and disgust-related primer (Disease: $z = 3.48$, $p < .001$; CD: $z = 2.21$, $p = .027$; C: $z = 0.417$, $p = .677$, Fig. 6, Supplementary Table 6). (ΔS-IgA) we found that the increase in S-IgA was significantly

Table 1
Descriptive statistics of the comparison of S-IgA [(mg/dl)/min] between Baseline and Sample 2 for the different primers.

Stimuli	n	$\bar{x}_{Baseline}$	$\bar{x}_{Sample2}$	$\sigma_{Basline}$	$\sigma_{Sample2}$	z	p
Aerosol	26	2.26	4.14	2.23	4.75	2.58	.010 *
Concealed Contagion	27	1.28	2.57	1.38	3.18	3.14	.002 **
Core Disgust	27	1.60	2.31	1.72	2.79	2.21	.027 *
Kontrolle	27	1.59	1.50	2.42	1.56	.79	.428 -

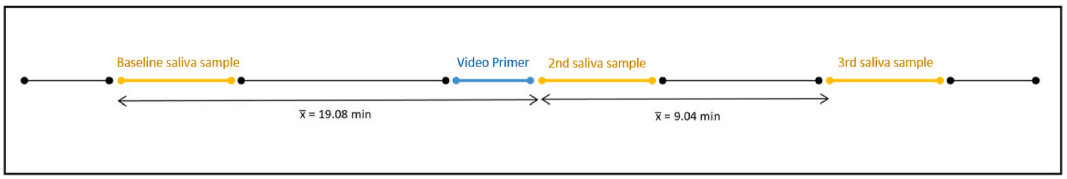

Fig. 2. Timing of the test session: Questionnaires (black), saliva samples (yellow) and video primer (blue) in relation to each other during the experiment. Average time between the starting points of the saliva samples is indicated below the chart. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

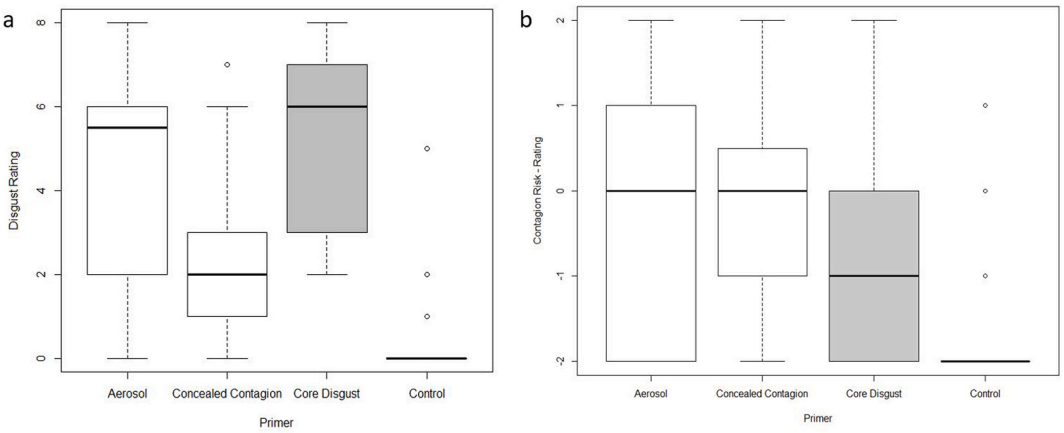

Fig. 3. Post-video evaluation of Disease (white), Disgust (grey) and Control Video Primers with regard to a) disgust rating ("*How strong was your feeling of disgust, antipathy and revulsion while watching the video?*"; 0 = *"not at all"*, 8 = *"completely"*) and b) contagion risk rating ("*If this was a real situation I would have become sick.*" 0 = *"completely incorrect"*; 4 = *"completely correct"*).

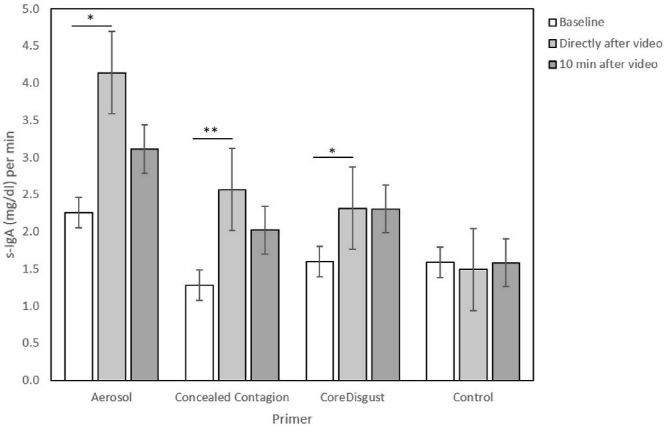

Fig. 4. Mean and standard error of the S-IgA concentration by primer at Baseline, Sample 2 and Sample 3. Significant changes are marked with asterisks (*p < .05; **p < .01).

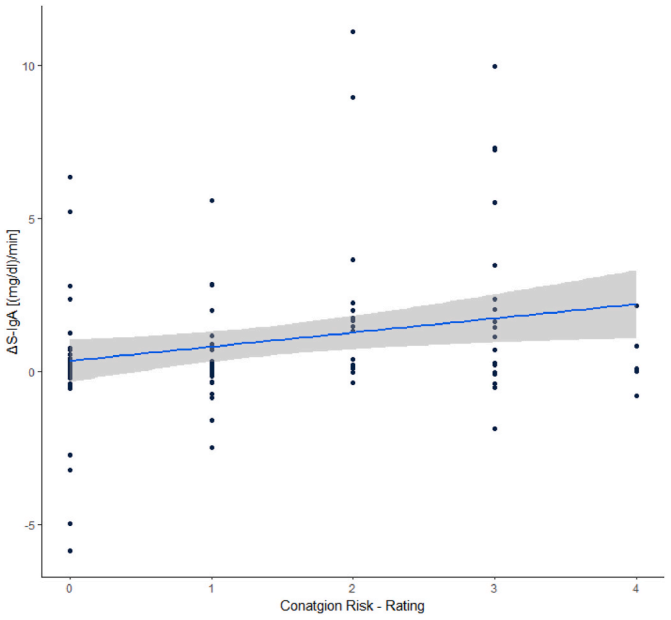

Fig. 5. Correlation between ΔS-IgA and contagion risk rating ("*If this was a real situation I would have become sick.*" 0 = *"completely incorrect"*; 4 = *"completely correct"*).

higher after the Disease Primer than after the Control Primer (U = 190.00, p = .004, p_a = .012, η = 0.153, Fig. 7). However, the S-IgA increase to the potentially contagious video content still did not significantly differ from the increase evoked by the Core Disgust Primer (U = 266.50, p = .091, p_a = .273, η = 0.053), which yet did not differ from the Control Primer (U = 240.50, p = .111, p_a = .333, η = 0.050).

5. Influence of trait disgust and perceived vulnerability to disease on S-IgA

Investigating the relationship between the S-IgA increase after the disease- or disgust-related primers and (1.) trait disgust, and (2.) VtD, we correlated the ΔS-IgA to the DS-R and pVtD scales and subscales. Since the control group showed no significant increase in S-IgA, we excluded these participants from the analyses. While we found no significant correlation between the ΔS-IgA and the total DS-R (r_s = −.187, p = .097) or the Core Disgust subscale (r_s = -.144, p = 203), we found a significant negative correlation with the Contamination Disgust subscale (r_s = -.239, p = .033, Fig. 8a).

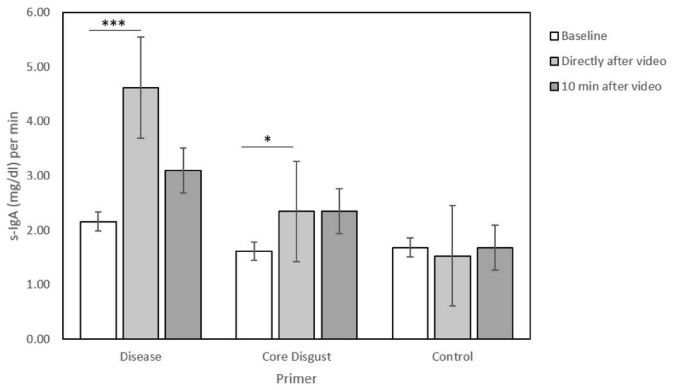

Fig. 6. Subsample of the participants who perceived the disease videos as potentially contagious, in comparison to the core disgust and control primers. Mean and standard error of S-IgA concentration at Baseline, Sample 2 and Sample 3. Significant changes are marked with asterisks (*p < .05; ***p < .001).

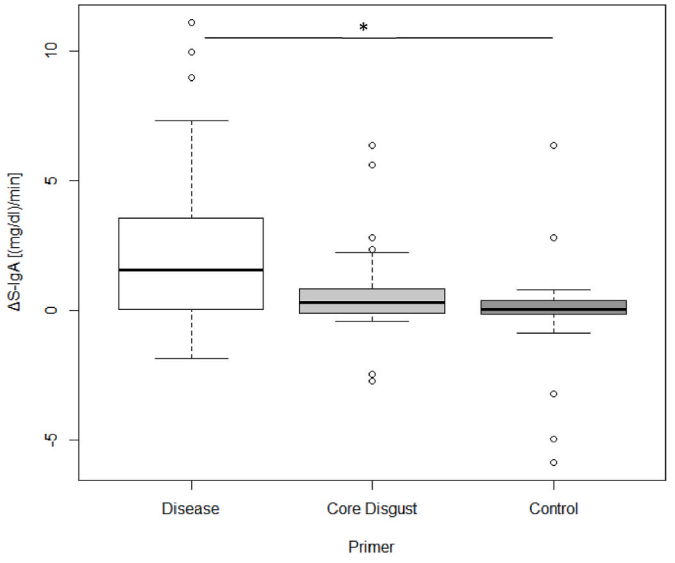

Fig. 7. Subgroup analysis of participants who perceived the disease-related primers as potentially contagious. ΔS-IgA concentration represents the increase between Baseline and Sample 2 (Sample 2 – Baseline). The significant difference is marked with an asterisk (*p < .05).

As the core disgust subscale mainly targets the items that are represented in the core disgust video, we also correlated the core disgust subscale separately to each primer. We found a significant correlation between the ΔS-IgA and core disgust in participants that were presented with the core disgust video (r_s = -.338, p = .046, see Fig. 8b), while the other groups showed no significant correlation (A: r_s = 0.158, p = .441; CC: r_s = -.206, p = .302). Furthermore, we did not find a significant correlation between the increase of S-IgA and the pVtD sum scale (r_s = -.039, p = .728) or its subscales Germ Aversion (r_s = 0.062, p = .583) and Perceived Infectability (r_s = -.104, p = .356).

6. Discussion

The goal of our study was to get a better insight into the interaction of the behavioral and the physiological immune system in disease- and disgust-related contexts. For this purpose, we created four sets of realistic video stimuli, which differed in their disease-association/contagion risk and overall disgust potential. We further measured the increase of S-IgA as evoked by the different videos and utilized various state and trait measures that revealed individual differences in disgust sensitivity and perception of disease threats. We found a significant increase of S-IgA secretion in our three disease- and disgust-related stimuli, which correlated positively with the perceived contagion risk and inversely with a trait measure of contamination disgust. On average, the S-IgA concentration increased by 83.15% after the Aerosol, by 100.63% after the Concealed Contagion and by 44.79% after the Core Disgust Primer. This was in so far unexpected since prior studies either reported only slight increases that occurred under specific circumstances (e.g., only in people with high trait disgust, Stevenson et al., 2015; or during a specific season, Brown et al., 2014) or even found a drop in S-IgA, particularly after disgust-evoking stimuli (Bosch et al., 2001; Stevenson et al., 2011, 2012). We can only speculate that the significant rise of S-IgA after all disease- and disgust-related videos was caused by the improved and supposedly more realistic stimulus material. On the one hand, we used short video clips showing real-life situations in combination with still pictures. On the other, our disease-related videos targeted respiratory pathogens and implied an increased risk of airborne contagion by the humans displayed. Previous studies used quite different disease-associated primers like x-rays or hospital hallways (Stevenson et al., 2015) or showed sick people who indicated no direct contagion risk (Brown et al., 2014). One may therefore assume that our videos more specifically activated defensive immune responses of the oral and nasal mucosae, which became evident in the significant rise of S-IgA in response to the two disease primers.

However, the extent of the actual increase (ΔS-IgA) induced by the two disease primers did not differ significantly from the control group, yet in the disease primer groups a significant rise from Baseline to Sample 2 could be documented which was not visible in the control primer (Fig. 4). Moreover, there was also no significant difference in the ΔS-IgA between the disease- and disgust-related primers. This lack of any significant differences in the primary analysis of the ΔS-IgA between primers could partially be caused by the data variance. Especially, the baseline sample showed a high variation between the four conditions, with the aerosol-group having the highest ($\bar{x}$ = 2.26 [(mg/dl)/min]; σ = 2.23) and the concealed contagion group with the lowest standard deviation ($\bar{x}$ = 1.28[(mg/dl)/min], σ = 1.38; also see Fig. 3). S-IgA is a very sensitive state measure and can be affected by many factors like psychological stress (Deinzer et al., 2000), physical stress (e.g.: sport, Keaney et al., 2018) and the consumption of certain foods and/or drinks (Kono et al., 2018). As our study was conducted during the COVID-19 pandemic we had to use an online test format, in which we were not able to control such factors up to the usual lab standard. We intentionally decided to use a between-subjects design for these 'at-home tests', since in a within-subject design with repeated tests over several days either the requirement of sample storage in participants' freezers over multiple days or the necessity to send the collected samples in multiple packages could have severely compromised sample quality. We are convinced that testing participants in a controlled laboratory environment with standardized relaxation periods for acute stress reduction and there also repeatedly with the different videos, i.e., in a within-subject design, would have significantly improved our data. The collection of stress related factors such as cortisol (Pawlow and Jones, 2005) as well as individual health parameters such as the level of B cells (Salvi and Holgate, 1999) might give an even better understanding of the variation in S-IgA concentrations and should be considered in a future between-subject design. Lastly, contributing to the heightened variance in the data, the present study design could have been slightly confounded. The participants filled in both the DS-R and the pVtD between the Baseline and the second (post-video) saliva sample and before watching the video primer. The statements of these questionnaires confront the participants with disgust-as well as disease-related situations, which might have had a promoting effect on S-IgA, even before the stimulation by the videos occurred. This seems plausible, since emotionally charged and disgusting statements alone have been found to already trigger disgust-related activation in the brain (Moll et al.,

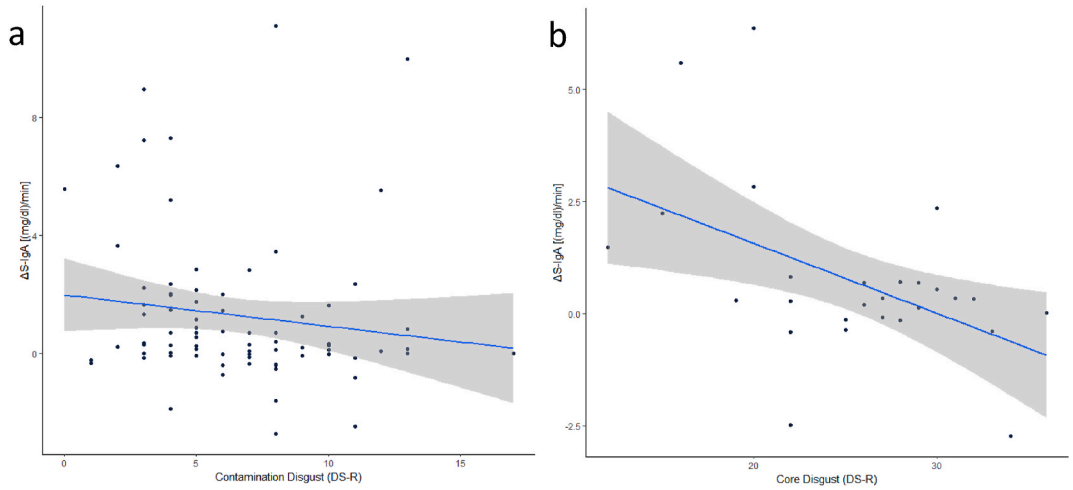

Fig. 8. Correlation between a) ΔS-IgA and Contamination Disgust (DS-R), excluding the control primer and b). ΔS-IgA and Core Disgust (DS-R) in participants that watched the core disgust stimuli.

2005).

Furthermore, we found that the perceived contagion risk associated with the videos correlated positively to the increase of S-IgA, implying that perceived contagion risk is an important factor in defensive immune responses. This assumption led us to perform a secondary analysis, from which we excluded participants that did not see the disease-related primers as a realistic contagion risk. Here we found a significant difference in ΔS-IgA between the disease-related and control primers, while the core disgust primer still did not significantly differ from neither the control nor the disease-related primer (Fig. 7). Lastly, it is important to mention that the data were collected during the Covid-19 pandemic. Although, the online tests were conducted from May to October 2021 during a period of relatively low case numbers, this pandemic of a potentially deadly respiratory disease, has altered the perception of sneezing and coughing people (Bouayed, 2022). Thus it might have had an influence on the extent of the S-IgA secretion, which needs to be ascertained by future post-pandemic replications. When looking into the interaction between BIS and PIS we could not find support for a co-occurrence of the two (high BIS activation = high physiological immune response), like Stevenson and colleagues found (2015). Contrary, our data show that the DS-R subscale contamination disgust correlated inversely with ΔS-IgA (Fig. 8a), and this was also the case for the core disgust DS-R subscale in participants that viewed the core disgust video (Fig. 8b). This would rather support the theory that the BIS and PIS compensate for each other (as also suggested by Fleischman and Fessler, 2011; Gassen et al., 2018). We therefore assume that people with a weaker PIS response, as reflected by a reduced ΔS-IgA after stimulation, might compensate this whenever confronted with a sick person by perceiving the situation as generally more disgusting, which also triggers increased avoidance, hence lowering contagion risk (Campbell et al., 2020; Dorfan and Woody, 2011). It is important to note that the findings of Stevenson et al. (2015) were based on a different disgust inventory than the one we used. They found a positive correlation between the change in S-IgA (after the presentation of their disgust-image set) to the pathogen subscale from the three dimensional disgust scale (Tybur et al., 2009, TDDS). They found no correlation to neither the DS-R contamination nor the core disgust subscale in their data.Nevertheless, these scales are close enough to compare the results. The pathogen subscale is defined as measure for the avoidance of infectious microorganisms (Tybur et al., 2009), the core disgust subscale measures sensitivity to offensiveness and threat of diseases and the contamination disgust subscale represents perceived threat of disease transmission (Olatunji et al., 2007). When comparing the items of the three questionnaires, the pathogen subscale (body fluids, rotten foods and animals) seems to combine the DS-R subscales of core disgust (disgusting food, animals, low hygiene) and contamination disgust (infectious body fluids, direct contact with pathogens). We speculate that the finding of their correlation (Stevenson et al., 2015), did not even represent the actual increase of S-IgA, but a relative value to a negative image set (anger-evoking: guns, domestic violence, personal distress), cannot be considered as reliable, since it may be severely statistically underpowered. Nevertheless, the absence of a correlation between ΔS-IgA and pVtD and its subscales in our study is coherent with the findings of Stevenson et al. (2015).

Furthermore, we found no significant correlation between our measures of state disgust (i.e., disgust questions from the Video evaluation and the mDES) and the S-IgA increase. This is coherent with findings of previous studies measuring S-IgA (Stevenson et al., 2015), IL-6 (Schaller et al., 2010), TNF-a/albumin and body temperature (Stevenson et al., 2012). Self-reported state disgust might not be the best way to measure the BIS in direct confrontations with disgust- and disease-related stimuli, as self-report of current state is very subjective and explicit, which makes it vulnerable to confounds like demand effects. Implicit methods such as behavioral computer tasks (e.g., to measure disease cue avoidance) might be a more suitable measure since they rather pick up implicit differences in the behavioral immune responses.

7. Limitations and future directions

Our data show activation of the PIS (higher concentration of S-IgA in saliva) following visual exposure to disease- and disgust-related stimuli. While one may speculate that the increase in S-IgA reflects a proactive immune response, that may prepare the organism for the upcoming pathogens associated with the sneezes and coughs, our study does not provide direct evidence for a heightened immunity in individuals who responded with increased S-IgA to the respective videos. However, such a proactive defense mechanism seems likely, since S-IgA in saliva plays an important role in immune exclusion and intracellular neutralization (Corthésy, 2013; Strugnell and Wijburg, 2010). Adding to that, heightened S-IgA is also discussed as a central biomarker of a reduced vulnerability to upper respiratory infections (Turner et al., 2021). Future studies have to further assess whether this increase in S-IgA represents the actual initiation of a preparatory immune response and thus reflects heightened immunity against the most common respiratory viruses even before the mucosae have come in contact with a pathogen. Nevertheless, we see our video priming experiment as a suitable method to further investigate how this interaction between the PIS and BIS is mediated by

the Neuro-Immune-Axis in future neuroimaging studies.

8. Conclusion

Our findings are indicative of an enhanced activation of salivary S-IgA secretion through visual perception of potentially contagious objects and people. We also found that people with a high contamination disgust secreted less S-IgA in such situations, which might be a hint for a compensating relationship between BIS and PIS. To get a further insight into the association of the BIS and PIS more research is needed. For future studies we suggest a better baseline control to reduce variance in data. This might be done by testing the participants in the controlled environment of a laboratory, by letting them relax for some minutes before the baseline sample and putting stricter restrictions on participants before testing (no spicy food on the day, no caffeine, etc.). We also suggest the implementation of additional implicit behavioral measures (e.g., implicit avoidance tasks) to get a better understanding of the behavioral response to the presented disease stimuli, since this aspect of the BIS may be insufficiently be represented by self-report questionnaires alone.

Declaration of interest

None.

Role of funding

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2022.100489.

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2.1.1 Supplementary material and methods of Chapter I

Supplementary

Supplementary Table 1: Sequence (Seq.), Kind, Length, Description and mean from Evaluation of Stimuli (EoS) of each stimulus within the Aerosol Disease Primer.

Seq.	Kind	Length (s)	Description	EoS
1	Photo	4	Women, facing camera, sneezing, aerosols visible	4.10
2	Video	27	Men, side profile, slow motion, sneezing, aerosols visible	4.04
3	Video	9	Women, facing camera, sneezing, teary eyes	2.36
4	Photo	4	Child, male, side profile, sneezing, aerosols visible	3.73
5	Video	8	Men, facing camera, sneezing	2.78
6	Video	3	Men, facing camera, sneezing, drool and aerosols visible	2.00
7	Video	4	Men, facing camera, sneezing	2.12
8	Photo	4	Men, side profile, sneezing, aerosol visible	4.03
9	Video	13	Women, side profile, slow motion, sneezing, aerosol visible	2.41
10	Photo	4	Men, facing camera, sneezing, a lot of visible aerosol	4.81
total		**80**	**Video was shown twice without any breaks**	**3.24**

Supplementary Table 2: Sequence (Seq.), Kind, Length, Description and mean from Evaluation of Stimuli (EoS) of each stimulus within the Concealed Contagion Disease Primer.

Sequence	Kind	Length (s)	Description	EoS
1	Photo	4	Men, facing camera, sneezing into right elbow	1.82
2	Video	27	Men, walking towards camera, sneezing into hand	1.77
3	Video	9	Women, side profile, sneezing into tissue, blowing nose	2.17
4	Photo	4	Women, facing camera, blowing nose into tissue	1.91

5	Video	15	Women, side profile, in bed, coughing/ blowing nose	1.57
6	Photo	4	Women, side profile, coughing into fist	1.86
7	Video	13	Men, facing camera, on couch, sneezing into tissue	2.23
8	Photo	4	Women, facing camera, blowing nose into tissue	2.47
total		**80**	**Video was shown twice without any breaks**	**1.98**

Supplementary Table 3: Sequence (Seq.), Kind, Length, Description and mean from Evaluation of Stimuli (EoS) of each stimulus within the Core Disgust Primer.

Sequence	**Kind**	**Length (s)**	**Description**	**EoS**
1	Photo	4	Mold on cream cheese container	4.16
2	Video	27	Several rats, dirty floor, dirty feet	3.76
3	Video	9	Several hornet larva	2.97
4	Photo	4	Cockroach	2.45
5	Video	9	Dog vomiting grass	2.06
6	Video	6	Mold on bread slices	3.69
7	Photo	4	Very dirty and overly messy room	3.5
8	Video	13	Strawberries molding, slow motion	3.78
9	Photo	4	Dead bat, with larva spilling out of open gut	4.93
total		**80**	**Video was shown twice without any breaks**	**3.48**

Supplementary Table 4: Sequence (Seq.), Kind, Length, Description and mean from Evaluation of Stimuli (EoS) of each stimulus within the Control Primer.

Sequence	**Kind**	**Length (s)**	**Description**	**EoS**
1	Photo	4	Bottom of plane flying over skyscrapers	0.68
2	Video	27	Drone flight over Chicago skyline	0.53
3	Video	9	Drone flight around skyscraper	0.64
4	Photo	4	Yellow entrance door to apartment building	0.64
5	Video	9	Pan over park with lake	0.37
6	Video	6	Birdseye view on fountain in the middle of a roundabout	0.80
7	Photo	4	Bike at bottom of a long bridge (over a river)	0.60
8	Video	13	Drone flight over skyscraper	0.94
9	Photo	4	Subway in train station	1.04
total		**80**	**Video was shown twice without any breaks**	**0.69**

Supplementary Table 5: All questions of the Relative-Feelings Questionnaire with phrasing of question in German.

	Question
1	Relative to before I feel more uneasy or apprehensive. (Ich fühle mich relative zu vorher unwohler oder beklommener.)
2	Relative to before I feel more inspired or creative. (Ich fühle mich relative zu vorher inspirierter und kreativer.)
3	Relative to before I feel more stressed or burdened. (Ich fühle mich relative zu vorher gestresster oder belasteter.)
4	Relative to before I feel more amused or exhilarated. (Ich fühle mich relative zu vorher amüsierter oder beschwingter.)
5	Relative to before I feel weaker or sicker. (Ich fühle mich relative zu vorher schwächer oder kränklicher.)
6	Relative to before I feel more optimistic or energetic. (Ich fühle mich relative zu vorher optimistischer und tatkräftiger.)

Supplementary Table 6: Subgroup analysis of participants who perceived the disease-related primers as potentially contagious. Descriptive statistics of the comparison of S-IgA [(mg/dl)/min] between Baseline and Sample 2 for the different primers.

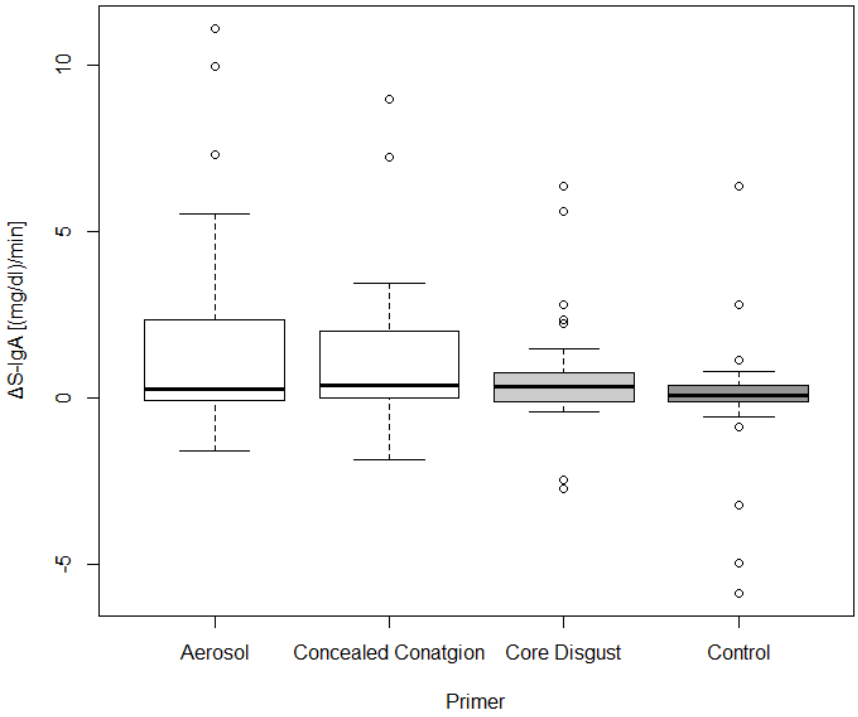
Stimuli	**n**	$\bar{x}_{Baseline}$	$\bar{x}_{Sample2}$	$\sigma_{Basline}$	$\sigma_{Sample2}$	**z**	**p**
Disease	28	2.16	4.62	2.16	4.88	3.48	>.001 ***
Core Disgust	27	1.60	2.31	1.72	2.79	2.21	.027 *
Kontrolle	24	1.69	1.53	2.49	1.61	.47	.677 -

Supplementary Table 7: Descriptive data on Sex differences in trait Disgust (DS-R and subscales), p-Values are based on Mann-Whitney-U test between the sexes.

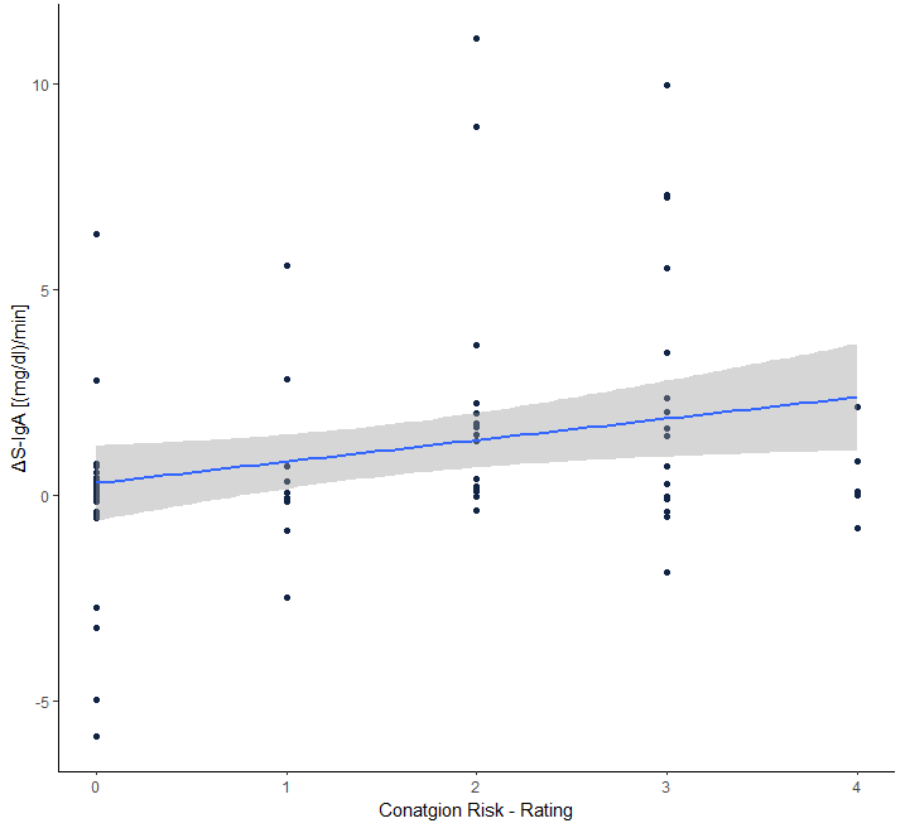
	Sum Disgust		Contamination Disgust		Core Disgust	
sex	f	m	f	m	f	m
N	68	48	68	48	68	48
Mean	34.71	29.77	6.68	6.40	28.03	23.38
p-value	.004		.503		>.001	

Supplementary Table 8: Descriptive data on Sex differences in trait vulnerability to disease (VtD), p-Values are based on Mann-Whitney-U test between the sexes.

	Sum VtD		Germ Aversion		Preceived Infectability	
sex	f	m	f	m	f	m
N	68	48	68	48	68	48
Mean	49.97	49.63	27.79	26.81	22.18	22.81
p-value	.877		.497		.606	



Supplementary Figure 1: ΔS-IgA concentration between Baseline and Sample 2, according to primer.



Supplementary Figure 2: Subgroup analysis of participants who perceived the disease-related primers as potentially contagious. Correlation between ΔS-IgA and perceived Contagion Risk (rating after the video).

2.2 Chapter II: SARS-CoV-2 specific sIgA in saliva increases after disease-related video stimulation

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SARS-CoV-2 specific sIgA in saliva increases after disease-related video stimulation

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Secretory immunoglobulin A (sIgA) in saliva is the most important immunoglobulin fighting pathogens in the respiratory tract and may thus play a role in preventing SARS-CoV-2 infections. To gain a better understanding of the plasticity in the mucosal antibody, we investigated the proactive change in secretion of salivary SARS-CoV-2-specific sIgA in 45 vaccinated and/or previously infected, generally healthy persons (18 to 35 years, 22 women). Participants were exposed to a disease video displaying humans with several respiratory symptoms typical for COVID-19 in realistic situations of increased contagion risk. The disease video triggered an increase in spike-specific sIgA, which was absent after a similar control video with healthy people. The increase further correlated inversely with revulsion and aversive feelings while watching sick people. In contrast, the receptor binding domain-specific sIgA did not increase after the disease video. This may indicate differential roles of the two salivary antibodies in response to predictors of airborne contagion. The observed plasticity of spike-specific salivary antibody release after visual simulation of enhanced contagion risk suggests a role in immune exclusion.

Since the initial outbreak of SARS-CoV-2 in Wuhan, China, in late 2019[1], COVID-19 evolved rapidly into a global pandemic, in part due to its airborne transmissibility that even further increased with emerging variants of concern. Its primary route of transmission through respiratory droplets and aerosols[2] suggests that the mucosal immune response in the oral and nasal cavities may be important for limiting viral infection. Within this context, secretory immunoglobulin A (sIgA) in saliva could play a significant role in preventing SARS-CoV-2 infections, as this is the most important immunoglobulin fighting pathogens in the respiratory tract[3]. SIgA is secreted by plasma cells adjacent to the mucosal epithelial cells[4]. It binds antigens and prevents their attachment to epithelial cells and is further involved in intracellular neutralization of viral replication, thus significantly contributing to immune exclusion[4]. Given these functions, sIgA may also have the potential of neutralizing SARS-CoV-2[3]. In fact, during the early stages of a SARS-CoV-2 infection SARS-CoV-2-specific IgA does not only dominate the humoral immune responses in serum, bronchoalveolar fluid and saliva, but it was also found to be more strongly correlated with the neutralization of the virus than the immunoglobulins M and G[5]. Furthermore, higher sIgA in saliva and nasal mucus has been associated with asymptomatic as opposed to symptomatic COVID-19-infections, which might also hint at its protective role against SARS-CoV-2[6,7]. Recent research findings further observed an increase in SARS-CoV-2-specific antibodies in saliva following intramuscular vaccination with the approved messenger ribonucleic acid (mRNA) vaccines developed by Pfizer/BioNTech (BNT-162b2) and Moderna (mRNA-1273)[8–10]. Also, the sIgA titer after vaccination seemed to be somewhat lower in people, who have not been previously infected with SARS-CoV-2[9,11]. Therefore, it would be interesting to know if the body has additional ways to transiently enhance the mucosal antibody level after vaccination, especially required in certain situations with heightened contagion risk that cannot be easily avoided.

For other viruses (e.g., influenza viruses), it has already been shown that the virus-specific antibody level in saliva can be enhanced on demand[12], if a person had already acquired the respective antibody repertoire through previous vaccination or infection. It thus seems plausible that following initial contact with COVID-19, either

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through infection or vaccination, the organism should be able to increase the release of SARS-CoV-2-specific sIgA in saliva whenever needed (e.g., after viral exposure). Interestingly, a number of psychoimmunological studies have recently demonstrated that actual pathogen exposure is not always obligatory to trigger a mucosal immune response. In fact, several immune markers in saliva and serum were found to respond proactively to the mere expectation of pathogen exposure, by showing an increase following visual stimulation with general disease-related content[13–16]. This was also the case for total sIgA in saliva, which increased after a video of people exhibiting typical symptoms of respiratory diseases (e.g., sneezing and coughing)[17]. Collectively, these findings led us to hypothesize that visual disease predictors, such as a video displaying people with respiratory symptoms, should trigger a proactive release of SARS-CoV-2-specific sIgA in a similar way in vaccinated individuals, and might thus transiently increase mucosal immunity temporarily even in the absence of the actual coronavirus. Such a proactive and virus-specific increase would be adaptive, given the high number of infected people in the population and the permanent risk of viral exposure.

To evaluate this we utilized an adapted test protocol from the study by Keller et al.[17]. The design comprised two within-subject test sessions, during which we measured SARS-CoV-2-specific sIgA and collected self-report state-measures of disgust and interoceptive feelings following a standardized test protocol (Fig. 1a). On two separate days, the participants either watched a disease video displaying people with respiratory symptoms or a control video with healthy people. Before and after the video, we measured SARS-CoV-2-specific sIgA to the spike and receptor-binding domain (RBD) antigens in saliva in order to assess their change from baseline to after the video. Based on our previous findings of a proactive increase in the total salivary sIgA following visual exposure with disease-related content[17], we expected the SARS-CoV-2-specific sIgA secretion to increase after the disease video displaying people with respiratory symptoms, but not after the control video.

In addition to that, our participants completed the *perceived Vulnerability to Disease Questionnaire*[18] and the revised *Disgust Scale*[19] some days before the first test took place. Further, state disgust and interoceptive feelings in response to the video were assessed during each test session according to the predetermined schedule[20,21]. It has previously been shown that both an increased disgust propensity and the acute feeling of revulsion may reduce contagion risk by proactively triggering the behavioral avoidance of an increased pathogen threat, which should in turn reduce the need for an enhanced physiological immune response[17,18,22]. Based on this evidence, we expected the different trait and state measures of disgust, disease propensity, and interoceptive feelings to negatively correlate with a proactive increase in SARS-CoV-2-specific sIgA in response to the disease video.

Results

We excluded the data of one participant, who was an outlier in all spike-specific and three of the RBD-specific sIgA samples (SARS-CoV-2-specific sIgA > two standard deviations above sIgA mean). The cohort used in this study was evenly male (n = 23) and female (n = 22), with an average age of 25.4 years (σ = 4.39). All participants were vaccinated against SARS-CoV-2, which was also indicated by their average IgG-titer in blood (mean = 993.33 BAU/mL; σ = 153.7) (see SI Table S1).

SARS-CoV-2-specific sIgA increase after disease-related stimulation

Spike-specific sIgA

In order to assess whether the disease video led to an increase in spike-specific sIgA, we performed a generalized linear mixed model (GLMM) with the factors *Video* (disease, control) and *Sample* (Baseline, Post-Video 1, Post-Video 2) as well as the random factor *Subject* with a gamma-log linked distribution as sIgA-data was left skewed. We found a significant main effect of *Video* ($F_{(1,264)} = 20.64$, $p = < .001$) and *Sample* ($F_{(2,264)} = 3.83$, $p = .023$) as well as a significant interaction between the two factors ($F_{(2,264)} = 6.16$, $p = .002$, find fixed coefficients in SI Table S5). In the post-hoc tests, this was reflected by a significant rise in spike-specific sIgA in the sample directly collected after watching the disease video (Post-Video 1) relative to Baseline ($z = -1.80$, $p = .036$, $\eta^2 = .72$), but not in the corresponding sample taken after the control video ($z = -.46$, $p = .648$). Additionally, spike-specific sIgA significantly declined from Post-Video 1 to Post-Video 2 after watching the disease video ($z = -2.56$, $p = .011$, $\eta^2 = .15$), but not after the control video ($z = -.12$, $p = .906$). Finally, we found that the samples collected at Post-Video 1 differed significantly between the two videos (disease > control: $z = -3.22$, $p < .001$, $\eta^2 = .23$), while the Baseline ($z = -1.59$, $p = .113$) and Post-Video 2 samples ($z = -1.25$, $p = .212$) did not (Fig. 1b).

We further ran an explorative analysis of *Video Order* as a covariate in the model, since it cannot be ruled out that the first video the participants had watched may have had an influence on sIgA secretion on the second test day. For full analysis see SI Results 2.4. In this analysis, we found a significant 3-way interaction between *Video*Sample*Video Order* ($F_{(2,258)} = 7.33$, $p < .001$). When data was split according to *Video Order*, post-hoc tests on ΔsIgA showed that the increase between Baseline and Post-Video 1 was only significantly higher after the disease video compared to control video, when participants saw the disease video first ($z = -2.71$, $p = .007$, $\eta^2 = .16$), but not when they saw the control video first ($z = 1.43$, $p = .153$).

Finally, we ran a confirmatory analysis of total sIgA, which had significantly increased in response to disease-related video content in our previous study[17].We found that total sIgA showed a similar response to the present disease video as spike-specific sIgA in that it showed a stronger increase after the disease than following the control video ($z = -1.75$, $p = .040$, $\eta^2 = .07$) (see SI Results 2.3). The $\Delta sIgA_{total}$ was further positively correlated with $\Delta sIgA_{spike}$ for the disease video (rho = .593, $p < .001$).

RBD-specific sIgA

In a second step, we analyzed the RBD-specific sIgA for changes induced by the disease video. In the GLMM we neither found a significant main effect of *Video* ($F_{(1,264)} = 3.10$, $p = .079$) nor of *Sample* ($F_{(2,264)} = .82$, $p = .444$), but there was a significant interaction between the two factors ($F_{(2,264)} = 6.79$, $p = .001$, find fixed coefficients in SI

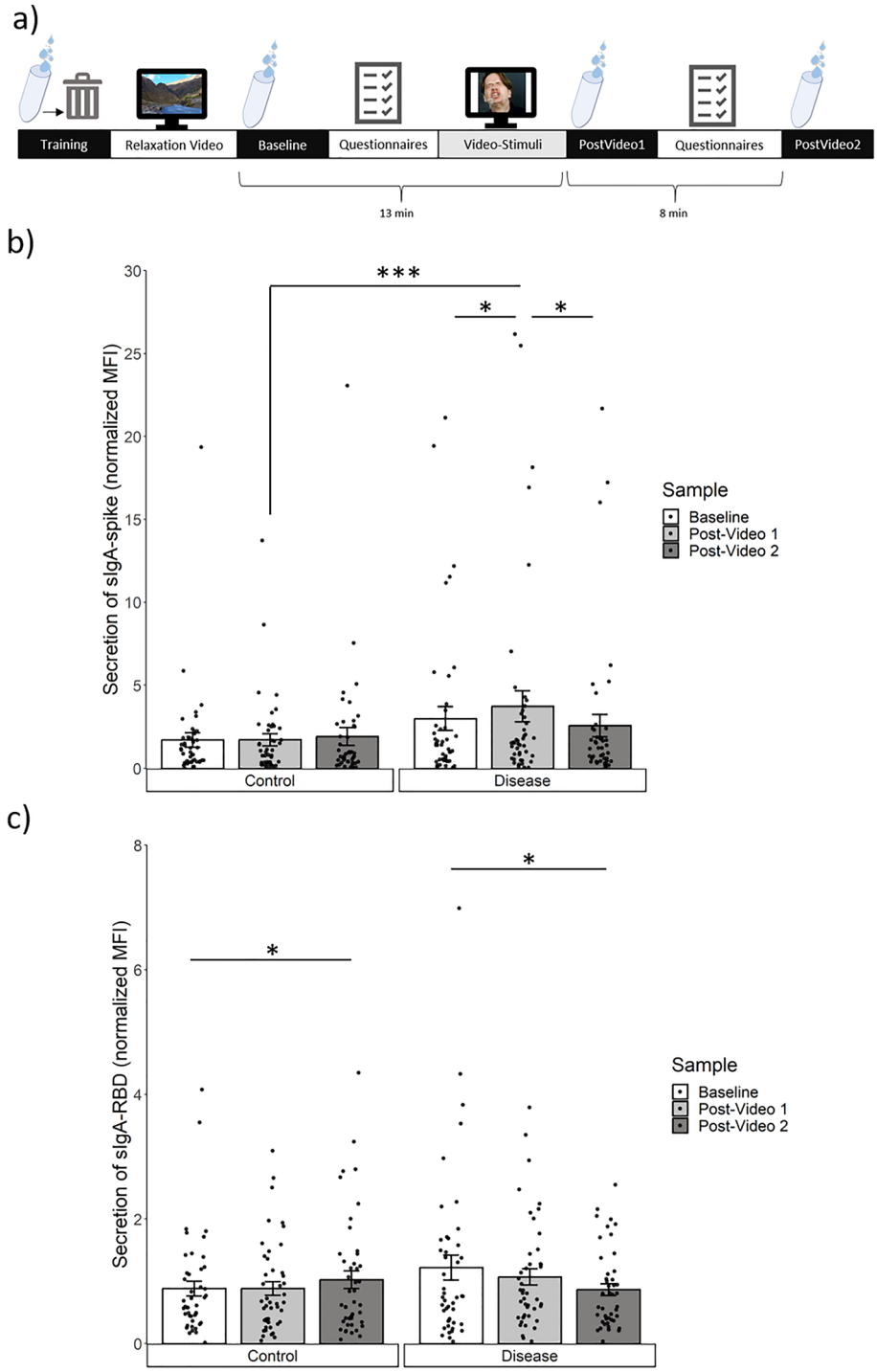

Figure 1. Antibody changes across test procedure. (**A**) Schematic of the test procedure: Temporal order of relaxation video and questionnaires (white), saliva samples (black), and stimulation video (gray) on each test day. Average time (in min) between the starting points of the saliva samples is indicated below the chart. (**B**) Spike-specific sIgA: Bar plot with mean, standard errors, and individual data points of the secretion rate at Baseline, directly after the video (Post-Video 1), and several minutes after the video (Post-Video 2). (**C**) RBD-specific sIgA: Bar plot with mean, standard errors, and individual data points of the secretion rate at Baseline, Post-Video 1, and Post-Video 2. Significant changes are marked with asterisks (*$p < .05$; ***$p < .001$), based on Wilcoxon signed rank test.

Table S6). Different from the spike-specific sIgA, the RBD-specific sIgA showed no significant rise from Baseline to directly after the disease video ($\Delta sIgA_{RBD}$: z = − .37, p = .714), and - similar to the spike-specific sIgA - also not after the control video ($\Delta sIgA_{RBD}$: z = − .04, p = .968). Instead, we found a trend-wise decline in the RBD-specific sIgA from Post-Video 1 to Post-Video 2 (z = − 1.95, p = .052, η^2 = .08), and also from Baseline to Post-Video 2 (z = − 2.18, p = .029, η^2 = .11) following the disease video. This indicated a continuous decrease in RBD-specific sIgA throughout the experimental session with the disease video. After the control video, we found a significant increase between Post-Video 1 and Post-Video 2 (z = − 2.07, p = .038, η^2 = .10), and also when comparing Baseline and Post-Video 2 (z = − 2.18, p = .029, η^2 = .11) (see Fig. 1c).

Trait disgust and perceived vulnerability to disease

The proactive increase in spike-specific sIgA in response to the disease video ($\Delta sIgA_{spike}$) neither correlated with the *Disgust Scale* (rho = .083, p = .294) nor with its subscales *Core Disgust* (rho = .005, p = .487) and *Contamination Disgust* (rho = .193, p = .102). Similarly, we found no significant relationship between $\Delta sIgA_{spike}$ and the total score of *perceived Vulnerability to Disease* (rho = − .172, p = .129) and also not with its subscales *Germ Aversion* (rho = − .108, p = .239) and *Perceived Infectability* (rho = − .190, p = .105).

State interoceptive and emotional reactions to the disease video

After having watched the given video, participants answered self-report questions on their feelings experienced during the disease video. We found that $\Delta sIgA_{spike}$ correlated inversely with the adapted *Respiratory Composite Score* (rho = − .299, p = .023, Fig. 2) of the *Interoceptive Feelings Questionnaire* (see also SI Table S4). The subscale of *Feelings in the Gut* only showed a trend-wise negative correlation with $\Delta sIgA_{spike}$ (rho = − .212, p = .081).

Additionally, the more state disgust participants indicated in the question "*How strongly did you feel disgust, antipathy and revulsion?*" after the disease video, the lower was their $\Delta sIgA_{spike}$ (rho = − .268, p = .037) (Fig. 3). In contrast, the average disgust rating of the screenshots from the disease video was not significantly correlated with $\Delta sIgA_{spike}$ (rho = − .159, p = .149).

Discussion

SIgA in saliva is an important part of the first line of defense against respiratory diseases such as COVID-19. So far, research on SARS-CoV-2-specific sIgA mainly focused on antibody titers in serum and saliva following vaccination, infection or passive transfer[23–25]. This study investigated the proactive change in secretion of SARS-CoV-2-specific sIgA to a video displaying people with respiratory symptoms typical for COVID-19. By this, we wanted to achieve a better understanding of the plasticity in the antibody response to situations with heightened contagion potential. We found the predicted increase in the spike-specific sIgA after the disease video, but not following a video with healthy people. The increase in spike-specific sIgA closely resembled the increase in total sIgA as shown in the confirmatory analysis and in line with our previous results[17]. This suggests that this SARS-CoV-2-specific component of sIgA may serve a similar proactive function in immune exclusion as previously described for total sIgA[4,17]. The $\Delta sIgA_{spike}$ further correlated inversely with state disgust and feelings

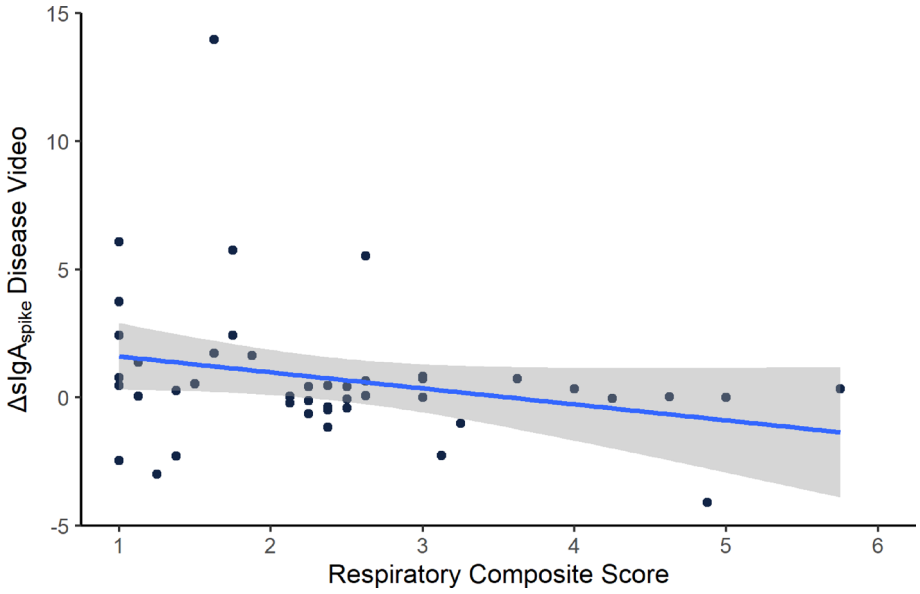

Figure 2. Interoceptive feelings in relation to spike-specific sIgA increase. Inverse correlation (*rho=-.299, p* = .023) between $\Delta sIgA_{spike}$ after the disease video and interoceptive feelings as measured by the *Respiratory Composite Score* (i.e., the combined score of items related to oral, contamination-associated and flu-like interoceptive feelings; see SI Table S4). Scatter plot with a linear model based on the data with 95% confidence interval in gray.

Figure 3. State disgust in relation to spike-specific sIgA increase. Inverse correlation (*rho=-.268, p* = .037) between $\Delta sIgA_{spike}$ and state disgust experienced during the disease video (Question: "*Please describe your emotions during the video: How strongly did you feel disgust, antipathy and revulsion?*"; 8 point-likert scale: 1 = "*I didn't feel like this at all*" to 8 = "*I felt completely like that*"). Scatter plot with linear model based on the with 95% confidence interval in grey.

of discomfort in the oral cavity and respiratory tract, suggesting a compensatory relationship between psychological and physiological defensive reactions to predictors of airborne contagion. In contrast, the RBD-specific sIgA did not increase after the disease video, but declined from Baseline to Post-Video 2, which may indicate rather differential roles of the two specific salivary antibodies in response to predictors of airborne contagion.

Antibodies against the spike protein of SARS-CoV-2 are particularly important as the spike protein includes the RBD, which is the main target of neutralizing antibodies[26]. Both spike- and RBD-specific IgA are not only found in serum of vaccinated or previously infected persons, but both antibodies occur in meaningful amounts in saliva as well[8–11]. We[17] and others[13] have previously demonstrated a transient, proactive increase in total sIgA initiated by visual cues of increased contagion risk. Such a quick rise in sIgA is possible as sIgA is constantly secreted into saliva even at baseline and can be rapidly upregulated by (para-)sympathetic[27] and mechanical stimulation[28]. The present observation of a significant rise in spike-specific sIgA by a median of 27.90% (Q_1: 17.33%, Q_3: 150.24%) following the ~ 8 min of mere visual experience of sneezing, coughing or otherwise sick persons, as well as its return back to baseline value shortly after the end of visual stimulation is consistent with these previous findings. The fact that this increase occurred in the absence of actual pathogen exposure indicates that the spike-specific sIgA could be part of a proactive immunological response that prepares the oral cavity for viral entry. We would therefore suggest that—similar to total sIgA– the spike-specific sIgA may be involved in immune exclusion rather than the actual neutralization of SARS-CoV-2[4]. This function would be quite adaptive, as heightened wild-type spike-specific sIgA in the mucosa has been observed to decrease the risk of infection even by the more contagious Omicron variant[29]. Apart from that, our data also showed that the RBD-specific sIgA did not follow the hypothesized pattern of a rise after the respiratory disease video, but - different from the spike-specific sIgA - declined over the course of the experiment. In contrast to anti-spike, RBD-specific antibodies have been shown to play a major role in neutralizing SARS-CoV-2[30–32]. Yet, they were found to be less abundant in saliva[26] and also less stable over time[33]. This is consistent with the observed baseline differences in the present study, with considerably higher spike- than RBD-specific sIgA (secretion rate: $mean_{spike\text{-}specific} = 2.36$, $SD_{spike\text{-}specific} = 3.23$; $mean_{RBD\text{-}specific} = 1.05$, $SD_{RBD\text{-}specific} = 1.01$). Also different from anti-spike, RBD-specific antibodies in saliva did not correlate well with RBD in serum[26]. The observed differences in the antibody response to the disease video might thus indicate some kind of compartmentalization of the mucosal immune response. In real life, the contagious respiratory droplets and aerosols of a sick person, that are emitted by sneezing, coughing, or even breathing, cannot be easily avoided in close social encounters. Thus, it may be adaptive to release the spike-specific sIgA as a proactive mechanism of immune exclusion, its release being already initiated in response to predictors of airborne contagion (here, the situations shown in the disease video). In contrast, the absence of an increase in RBD-specific sIgA in response to the visual disease predictors suggests, that the release of neutralizing antibodies may only be increased once the mucosae have come in contact with the viral antigen. This would then rather reflect a reactive immune response of the RBD-specific sIgA to the specific pathogen. The parallel decline of spike- and RBD-antibodies after the offset of the disease video, i.e., from Post-Video 1 to Post-Video 2, might then be explained by the discontinuation of the visual predictor (in case of anti-spike) and by the absence of a factual virus-mucosae contact (in case of anti-RBD, and supposedly also anti-spike), which

would render an immune response unnecessary. However, future studies have to further address these speculations, especially those regarding the nature of the anti-RBD response and the proposed compartmentalization of the mucosal immune response.

From our present finding we cannot unequivocally infer that the mucosal immune response to the respiratory disease video will always follow the observed pattern. Even though, the shape of the spike-specific sIgA strongly resembled the one observed for total sIgA in our previous study[17], and in the confirmatory analysis of the present study, our participants were nevertheless tested during the ongoing COVID-19 pandemic. Most tests of the current study took place during the first and second Omicron wave in Northern Germany. Although all participants were vaccinated, the new Omicron variant and its various subvariants created a context of heightened contagion risk for COVID-19, as seen by the large number of breakthrough infections among vaccinated individuals in 2022[34]. For other viral respiratory pathogens like influenza, a high risk context (e.g., the flu season) has previously been shown to be linked to a surge in total sIgA to visual disease predictors, while a low risk context was not [13]. It remains to be ascertained in the future, how people would respond to our disease video once SARS-CoV-2 has become endemic and COVID-19 morbidity and mortality is significantly reduced.

Apart from the increase in spike-specific sIgA on the group level, there was also considerable variance in the extent of the $\Delta sIgA_{spike}$. Such interindividual differences in the proactive immune response have previously been explained by a compensatory relationship between physiological immune and behavioral avoidance responses. The associated feeling of disgust may thereby facilitate avoidance of disease cues, which in turn reduces the need to prepare the immune system for potential pathogen contact[17,35,36]. In line with these prior findings, we found an inverse correlation of $\Delta sIgA_{spike}$ with the *Composite Respiratory Score* from the *Interoceptive-Feelings Questionnaire*[20]. Interoception is a wide construct that not only includes the awareness/feeling of bodily sensations, but also the interpretation of such information and the consequential behavior[37]. Thus negative, oral and contamination-related interoception such as an itch in the throat, the urge to cover your mouth or the feeling of flu-like symptoms during the video can be seen as proactive interoceptive responses that may trigger avoidance of their generators. We did not find a significant correlation between $\Delta sIgA_{spike}$ and the *Composite Gut Score*, and the respective score was lower than the *Composite Respiratory Score* (see SI Results 2.5). This indicates that acute bodily sensations may be specific for the category of disease cues and the associated pathway of contagion. COVID-19 is mainly a respiratory disease[2], and airborne transmission is the dominant route of contagion[38], which is why the present disease video, that focused on respiratory symptoms, may have specifically triggered sensations in the respiratory pathway. In a similar vein, we observed an inverse correlation of $\Delta sIgA_{spike}$ and self-reported state disgust experienced during the disease video. While this also fits with the hypothesis of a compensatory relationship between behavioral and physiological responses to enhanced contagion risk[39], this relationship has not been found with total sIgA[15,17,40]. We can only speculate that either the spike-specific sIgA surge is uncoupled from total sIgA in saliva, which is rather unlikely since our confirmatory analysis showed a correlation between the two, or that the current disease video induced a sufficient variation in both the state disgust rating and the physiological immune response of the 45 participants, rendering this correlation more likely. However, since all correlations were rather small (< 0.3), a replication is needed. What is nevertheless noteworthy is the complete absence of an association between $\Delta sIgA_{spike}$ and the trait measures of disgust and disease vulnerability. Like in our previous study[17], these trait measures may not be indicative of the capacity of the mucosal immune system to proactively release antibodies in response to predictors of contagion.

On an intraindividual level we should note that the baselines of spike-specific and RBD-specific sIgA showed a slight variation between disease and control video, although not significant. As a highly variable parameter that responds to even small changes in the mouth (e.g., chewing[28], food or drink[41]), sIgA baseline differences even within the same person (when tested on different days) were to be expected. Different from caged test animals, daily stressors, differences in food ingestion etc. could not be controlled in our human volunteers. Although we tested only nonsmokers, instructed our participants to refrain from eating 2 h before the test and to refrain from taking medication or food additives for at least 48 h before the test, we had no chance to control everything in their daily life.

In addition to that, we also explored the within-subject design for possible order effects and observed a significant three-way interaction between the factors *Video, Sample* and *Video Order* in the spike-specific sIgA, which also became evident in the analysis of total sIgA (see SI Results 2.4.3). The increase of spike-specific sIgA during the disease video thereby only differed significantly from the change during the control video, if participants experienced the disease video first. We can only speculate that this may have been caused by an interpretational bias. Interpretational biases in (visual) cognition have already been found to alter emotional reactions and may possibly also affect the associated physiological responses[42,43]. The present study was explicitly advertised as a project that assessed immunological responses to SARS-CoV-2. This advertisement might have led to certain expectancies that should have particularly affected the naïve test day, when everything was new and participants expected to contribute in a research project on SARS-CoV-2. As a result, watching the disease video on the naïve test day might have induced a potent effect on sIgA release, while even the control video might have been perceived as more salient on the first day, also given common knowledge that even asymptomatic persons can transmit the virus[44]. In an explorative comparison we found that participants perceived the control video as more disgusting when they saw the control video first (see SI Results 2.4.4.). Then, on the second day, the reduced relative rise during the disease video may also be explained an expectation effect. After having watched the control video on the first day, participants most likely expected to receive a more disease-associated stimulation on day 2, which would fit with the observation of the already higher spike-specific sIgA baseline concentration on the second day in the group of participants that watched the control video first (see SI Fig. S3b). However, since this order effect was analyzed post-hoc, we can only speculate in this regard. Future studies will be necessary to assess the influence of interpretational biases and expectancy effects on proactive immunological responses, which might be caused by prior experience, task order or conditioning effects. Finally, it is important to note that our

pre-registered study design was counterbalanced for task order and we also found a significant increase in both spike-specific and total sIgA after the disease video in the total group, regardless of video order.

This is the first study that demonstrated the plasticity of salivary antibody levels against SARS-CoV-2 in response to a visual simulation of heightened airborne contagion potential. It shows that spike-specific sIgA can be released on demand, in response to unequivocal disease cues and at one of the crucial viral entry points, the oral mucosae. Nevertheless, several important questions still remain unanswered. First, the virus neutralizing capacity of the released spike-specific sIgA was not tested, and therefore the actual immunological advantage of this proactive response remains to be proven. Second, the meaning of the decline in RBD-specific sIgA could only be indirectly attributed to the absence of a factual viral exposure, and the interpretation of this finding thus rather represents a hypothesis than an inference. Again, further evidence is needed to probe the theory that neutralizing RBD-antibodies require mucosal contact with the virus to be released. Third, as already indicated above, this study was conducted during the COVID-19 pandemic in a phase of heightened contagion risk, i.e., Omicron waves. In addition, the participants had quite recently received a vaccination, which was also reflected by the relatively high average blood IgG-titer that may be associated with a potent mucosal antibody reservoir. For these reasons, our results might be quite specific for the pandemic situation and a population with sufficient immunity. It thus needs to be ascertained in the future, whether these results of our intervention can be replicated in people with dwindling antibody levels and outside of the pandemic context. Finally, our study does not answer the question, whether less obvious markers of respiratory diseases (e.g., changes in skin coloration, increased sweating) that may be carried by otherwise asymptomatic people, and which might be unconsciously perceived[45], also have the potential to activate this route of the mucosal immune defense. In that context, the associated neural pathway would also be of increased interest.

Materials and methods

Participants

In a within-subject design we confronted the participants with two different videos (disease and control) on two different test days. We recruited 46 participants (24 m/22 f) on the university campus, through online advertisements, and via social media. We only invited healthy individuals to participate, who were between 18 and 35 years old, and who had been vaccinated at least twice with one of the mRNA-vaccines against SARS-CoV-2. Female participants were only included, if they used hormonal contraception containing ethinylestradiol (to ensure a homogeneity of steroid hormones within the female participants). Data collection took place from February to April 2022. Participants received a financial reward of 35 Euros. We obtained informed consent from all participants and the procedure was approved by the local ethics committee *"Ethikkommission der Ärztekammer Hamburg"* (PV3938) and conformed with the Declaration of Helsinki.

Stimuli

During the two test sessions, participants were primed with either a disease or a control video. The order of the videos was counterbalanced. The disease video was a 5 min video displaying short clips of people with symptoms of respiratory diseases, e.g., sneezing and coughing, as well as blowing their nose and lying sick in bed (Fig. 4a). The control video was matched to the disease video and showed healthy people in similar environments (Fig. 4b). User licenses for videos were obtained from the respective online platforms (iStock, pexels, etc.). For detailed information see SI Tables S2 & S3.

Procedure

Prior to invitation for test sessions, all participants completed an online survey on demographic data and medical history. This survey also included the revised *Disgust Scale* (DS-R)[19] as well as the *perceived Vulnerability to Disease Questionnaire* (pVtD)[18].

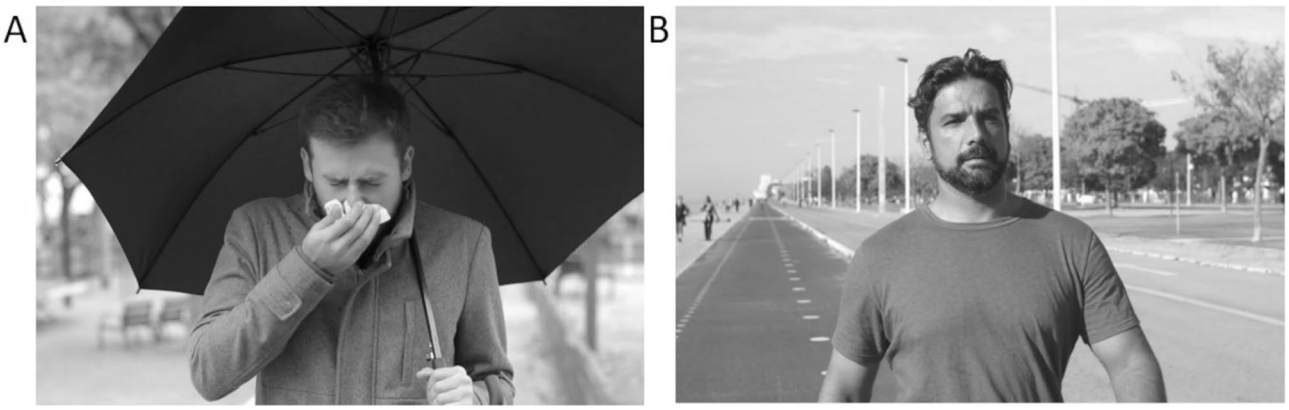

Figure 4. Examples from the two stimulus sets used in the videos. (**A**) Exemplary screenshot from the disease video (www.istockphoto.com; by Antonio Guillem); (**B**) Exemplary screenshot from the control video (www.pexels.com; by Kampus Production).

The two test sessions were conducted at the Institute for Animal Cell and Systems Biology, Universität Hamburg in the afternoon (between 12 and 5 pm, and at least 24 h apart [$\bar{x} = 5.56$ days; $\sigma = 4.39$ days]). In the beginning of the first test session, participants were informed about the general purpose of the study, the opportunity to abort data collection at any time, as well as aspects concerning anonymity and safety. Upon arrival, participants also gave an initial practice saliva sample that was discarded afterwards. Subsequently, they watched a 5 min relaxation video showing waterfalls and nature scenery, while listening to relaxing music. The relaxation video was intended to reduce anticipatory stress and anxiety in the unknown test environment. This was followed by the Baseline saliva sample and participants providing additional demographic data on aspects such as age, sex, and current state of health. Here, they also reported, whether they had been exposed to any stressors, such as smoking, sports, alcohol within the last 48 h, as well as any current and previous diseases, before moving on to the *Mood Scale*[46]. The *Mood Scale* was included to control for potential mood differences between test days. It was followed by one of the two videos, to which the participants were randomly assigned on the first test day (the other video was shown on the second test appointment). The second saliva sample was taken immediately after the end of the video (Post-Video 1). After filling out further questionnaires related to attention, emotion[21] and somatic feelings during the video stimulation, participants were finally asked to give the third and last saliva sample (Post-Video 2).

At the end of the disease video session, we finally measured participants' IgG-Titer in the blood (BAU/mL) utilizing the VitaLab LS-1100 diagnostic device with the dry fluorescence Immunoassay Test Kit.

Saliva samples

During each test session three saliva samples were collected at Baseline, Post-Video 1 and Post-Video 2 (Fig. 1a). Participants filled the three microcentrifuge tubes (2 mL) by passive drooling. The experimenter stopped the time it took to fill up a tube. Afterwards the samples were weighed and frozen at − 80 °C. After being frozen for at least 24 h the samples were thawed and deactivated (centrifuged, mixed with tri-n-butyl phosphate and Triton-X100), as per protocol in Becker et al.[8]. Salivary IgA titers were analyzed using MULTICOV-AB, a multiplex SARS-CoV-2 immunoassay[8,47] to determine SARS-CoV-2 antigen-specific antibody titers and an IgA ELISA (LDN Immunoassays #SA E-6800R) to determine total salivary IgA. Both protocols were performed either according to the manufacturer's protocol (IgA ELISA) or as previously described (MULTICOV-AB), whereby each saliva sample was assayed twice and the mean of the two measurements was used for analysis. All saliva analysis were performed blinded, although all samples from a single individual were included on the same plate. The values of the Sars-CoV-2 specific sIgA were normalized to nucleocapsid antibodies. Normalization was performed to standardize and remove as many environmental effects as possible. This is often necessary with saliva due to the inherent material itself. Saliva is not an ideal matrix due to the number of individual differences present (e.g. viscosity, bacterial/yeast contamination), all of which affect the ability to generate accurate measurements from it. While normalization would usually involve the use of reference samples, unfortunately saliva reference samples were unavailable due to the type of material itself, making this type of normalization impossible. Similarly, normalizing to reference serum samples would not have been ideal, as our normalization values would have then been resulting from a completely different sample matrix. We therefore chose to normalize between analytes in a sample as is done for other molecular biology techniques such as RT-PCR. This enabled us not only to have a direct evaluation of the change in antibodies generated/detected (e.g. increase in spike production), but also to normalize our samples regardless of their individual differences. By using Nucleocapsid antibodies as an effective quality control from sample to sample, we could assess antigen-specific changes in antibody levels within each sample.

Data analysis

After data collection, but before data analysis, we preregistered the planned analysis (https://osf.io/br3xm/). For data analysis we calculated the sIgA secretion rate, which is determined by multiplying the absolute sIgA Measure (normalized ($\frac{SpikeorRBD}{Nucleocapsid}$) mean fluorescence intensity (MFI)) with the flow rate ($\frac{mL}{min}$) (i.e., secretion rate = normalizedMFI $*$ $\frac{mL}{min}$). All data were tested for deviation from a normal distribution using the Kolmogorov–Smirnov test with a statistical threshold of $p < 0.05$. Since all KS-tests were significant, we used non-parametric post-hoc tests. All data analysis was conducted with IBM SPSS (Version 29.0.0.), figures were generated using R Studio (Version 4.2.3).

We assessed whether the increase in sIgA secretion rate (spike- and RBD-specific) was affected by the category of the videos. For this, we planned to utilize a 2 × 3 general linear model for repeated measures (GLM) with *Video* (disease and control video) and *Saliva Sample* (Baseline, Post-Video 1, and Post-Video 2) as within-subject factors. However, during the analysis process, we decided to use a Generalized Linear Mixed Model (GLMM), which allows adding random effects of intercept as well as of slopes. As the distribution of sIgA data has a left skew and no negative values we decided to use a gamma distribution with log link, with sIgA secretion rate as Target, *Video* & *Sample* as Fixed Effects and Interactions and a random intercept of *Subject*. We further utilized robust covariances to accommodate for possible violations of model assumptions (the SPSS syntax file is uploaded under https://osf.io/br3xm/). The results of the originally planned GLM can further be found in the Supplement (see SI Results 2.6). As post-hoc tests, we conducted Wilcoxon-signed-rank tests. In addition, we employed Spearman-correlations to assess the association between the increase of sIgA (ΔsIgA = Post-Video1—Baseline) following the disease video and the questionnaire scores. Post-hoc test and correlations regarding our directed hypotheses were conducted one-sided.

Data availability

The data used for the analysis that support the findings of this study are available on OSF.io (https://osf.io/br3xm/).

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Author contributions
E.D & J.K.K. initiated the research and further set the study conception, design and material. A.H., J.K. and J.K.K. collected the data. A.D. designed and supervised the laboratory measurements of salivary antibodies. A.D., Je.G. & Jo.G performed the laboratory analyses. J.K.K. & E.D. statistically analyzed the data. N.S.M. obtained funding. The first draft of the manuscript was written by J.K.K. with support of E.D. All remaining authors provided feedback on the manuscript draft.

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Competing interests
The authors declare no competing interests.

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2.2.1 Supplementary material and methods of Chapter II

1. Supplementary Material & Methods

1.1 Participants

Table S1: Information about participants including Sex (f=female, m=male), Age (years), SARS-CoV-2 vaccination (Dose 1-3) and infection background of participants (reported cases confirmed by PCR)

ID	*Sex*	*Age*	*1st Dose*	*2nd Dose*	*3rd Dose*	*Previously infected*
1	m	24	Vaxzevria	BNT-162b2	BNT-162b2	No
2	f	21	BNT-162b2	BNT-162b2		Yes
3	m	30	mRNA-1273	mRNA-1273		Yes
4	f	25	BNT-162b2	BNT-162b2	BNT-162b2	No
5	m	25	BNT-162b2	BNT-162b2	mRNA-1273	No
6	m	21	BNT-162b2	BNT-162b2	BNT-162b2	No
7	m	22	BNT-162b2	BNT-162b2	BNT-162b2	No
8	m	29	Vaxzevria	BNT-162b2	BNT-162b2	Yes
9	f	26	BNT-162b2	BNT-162b2	BNT-162b2	No
10	m	25	BNT-162b2	BNT-162b2	BNT-162b2	No
11	m	20	BNT-162b2	BNT-162b2	BNT-162b2	No
12	m	26	BNT-162b2	BNT-162b2	BNT-162b2	No
13	f	24	BNT-162b2	BNT-162b2	BNT-162b2	No
14	f	20	BNT-162b2	BNT-162b2		Yes
15	f	28	BNT-162b2	BNT-162b2	BNT-162b2	No
16	m	26	mRNA-1273	mRNA-1273		Yes
17	f	23	mRNA-1273	mRNA-1273	mRNA-1273	Yes
18	m	24	BNT-162b2	BNT-162b2	BNT-162b2	Yes
19	m	32	mRNA-1273	mRNA-1273	BNT-162b2	No
20	f	18	BNT-162b2	BNT-162b2	BNT-162b2	No
21	f	24	mRNA-1273	mRNA-1273	BNT-162b2	No
22	f	24	BNT-162b2	BNT-162b2	BNT-162b2	No
23	m	24	BNT-162b2	BNT-162b2	BNT-162b2	No
24	m	27	BNT-162b2	BNT-162b2		Yes
25	m	19	BNT-162b2	BNT-162b2	BNT-162b2	No
26	f	20	BNT-162b2	BNT-162b2		No
27	m	30	BNT-162b2	BNT-162b2	mRNA-1273	No
28	f	33	BNT-162b2	BNT-162b2	mRNA-1273	No
29	m	29	BNT-162b2	BNT-162b2	BNT-162b2	No
30	m	20	BNT-162b2	BNT-162b2	BNT-162b2	No
31	f	20	BNT-162b2	BNT-162b2	BNT-162b2	No
32	f	30	BNT-162b2	BNT-162b2	mRNA-1273	No
33	f	28	BNT-162b2	BNT-162b2	BNT-162b2	Yes
34	f	23	BNT-162b2	BNT-162b2	BNT-162b2	No
35	m	35	Jcovden	mRNA-1273	mRNA-1273	No
36	m	28	BNT-162b2	BNT-162b2	BNT-162b2	No

Continuation of Table S1: Information about participants including Sex (f=female, m=male), Age (years), SARS-CoV-2 vaccination (Dose 1-3) and infection background of participants (only cases confirmed by PCR)

Subject	*Sex*	*Age*	*1st Dose*	*2nd Dose*	*3rd Dose*	*Previously infected*
37	m	28	mRNA-1273	mRNA-1273	BNT-162b2	No
38	f	24	BNT-162b2	BNT-162b2	mRNA-1273	No
39	f	21	BNT-162b2	BNT-162b2	BNT-162b2	No
40	f	31	BNT-162b2	BNT-162b2	BNT-162b2	No
41	f	20	BNT-162b2	BNT-162b2	BNT-162b2	No
42	f	26	BNT-162b2	BNT-162b2	BNT-162b2	No
43	m	33	BNT-162b2	BNT-162b2	BNT-162b2	No
44	f	23	Jcovden	BNT-162b2	BNT-162b2	No

1.2 Video material

Table S2: Content of the disease video. Sequence (Seq.), length and description of the video content shown.

Seq.	*Length (s)*	*Description*
1	3	Man, facing camera, sneezing
2	8	Woman, side profile, slow motion, sneezing, aerosols visible
3	12	Woman, sick in bed, coughing into tissue
4	10	Woman, facing camera, slow motion, sneezing, aerosols and snot visible
5	8	Woman, facing camera, sneezing 3 times, teary eyes
6	22	Man, facing camera, walking with an umbrella, sneezing into tissue 4 times, red nose
7	4	Man, facing camera, slow motion, sneezing, aerosols visible, blurry
8	8	Man, facing camera, sneezing 3 times, teary eyes
9	14	Woman, lower half of face visible, sitting outside, cleaning nose with tissue
10	13	Man, facing camera, sneezing 4 times, drool visible, teary eyes
11	7	Woman, side profile, sneezing into tissue and cleaning nose
12	4	Man, facing camera, sneezing, tears and a lot of drool visible, teary eyes
13	7	Woman, facing camera, sneezing 3 times, teary eyes
14	3	Woman, facing camera, sneezing, teary eyes
15	12	Man, sitting outside, side profile, lower half of face visible, sneezing and coughing 2 times

Continuation of Table S2: Content of the disease video. Sequence (Seq.), length and description of the video content shown.

Seq.	*Length (s)*	*Description*
16	8	Man, side profile, slow motion, sneezing, a lot of drool and aerosols visible
17	6	Woman, facing camera, sneezing, drool visible, teary eyes
18	8	Man, facing camera, sneezing 2 times, aerosols and tears visible, teary eyes
19	6	Man, side profile, slow motion, outside, Sneezing, aerosols visible
20	4	Man, facing camera, slow motion, outside, sneezing, aerosols visible
21	3	Man, facing camera, slow motion, outside, sneezing, aerosols and snot visible
22	10	Woman, facing camera, sitting on bed, sneezing 6 times
23	7	Man, facing camera, slow motion, coughing
24	11	Man, facing camera, slow motion, sneezing, snot visible
25	4	Woman, facing camera, sneezing 2 times, teary eyes
26	13	Man, facing camera, sitting on couch, visibly sick, sneezing into tissue, drinking tea
27	11	A sequence of short clips showing 3 women and 6 man, facing camera, sneezing
28	6	Woman, side profile, slow motion, sneezing into tissue
29	3	Man, side profile, sneezing 2 times, aerosols visible
30	23	Man, side profile, slow motion, sneezing, a lot of aerosols visible
31	4	Man, facing camera, sneezing, tears and a lot of drool visible, teary eyes
32	7	Woman, facing camera, sneezing 2 times, aerosols visible
33	16	Man, facing camera, slow motion, sneezing
34	13	Woman, side profile, sick in bed, sneezing into tissue 2 times
total	**299**	

Table S3: Content of the control video. Sequence (Seq.), length and description of the video content shown.

Seq.	*Length*	*Description*
1	13	Woman, facing camera, looking at camera
2	10	Man, sitting on couch, talking on his phone
3	10	Woman, lying in bed
4	14	Man, facing camera, looking at the camera
5	6	Woman, facing camera, smiling at the camera
6	8	Man, top half of body visible, walking outside
7	18	Woman, lying in bed, stretching arms
8	8	Man, facing camera, looking around
9	11	Woman, side view, entire body visible, typing on the computer, smiling
10	9	Man, top half of body visible, typing on his phone, scratching his nose
11	11	Woman, facing camera, looking and smiling at camera
12	12	Man, facing camera, looking around, scratching his cheek
13	20	Woman, side view, entire body visible, outside, leaning against a railing, drinking out of a cup
14	14	Man, side view, entire body visible, sitting on a bed, typing on computer
15	4	Woman, facing camera, looking at the camera
16	7	Man, facing camera, looking and smiling at camera
17	16	Woman, side view, upper half of body visible, sitting on a bed, typing on computer
18	16	Man, facing camera, lying in bed with closed eyes, moving around
19	21	Woman, side view, entire body visible, sitting on an armchair, reading a book
20	10	Man, front view, entire body visible, sitting on a bed, typing on computer
21	5	Woman, facing camera, looking at the camera
22	6	Man, birds view, upside down, lying on the floor, listening and moving to music
23	21	Woman, front view, upper half of body visible, leaning against kitchen counter, looking at phone, drinking out of a cup
24	13	Man, front view, upper half of body visible, walking through a glass building
25	10	Woman, side view, entire body visible, lying on bed, reading a book
total	**293**	

1.3 Description of Questionnaires

Table S4: Interoceptive Questionnaire that follows the Post-Video 1 sample (modified and adapted questionnaire of Kupfer et al. (2021); two subscales were formed by averaging corresponding questions)

Composite score	***Please rate the following statements:*** **(options: *1-not at all, 2, 3, 4, 5, 6, 7-very strongly*)**
Gut	*I felt nauseous during the video.*
Gut	*I felt like I could vomit during the video.*
Gut	*I felt a physical sensation in my stomach, during the video.*
Respiratory	*I felt a physical sensation in my throat.*
Respiratory	*I felt an increased saliva secretion during the video.*
Respiratory	*I felt an urge to cover my mouth and nose with my hand during the video.*
Respiratory	*I had a feeling of contamination during the video.*
Respiratory	*I felt unclean during the video.*
Respiratory	*I felt the urge to wash my hands during the video.*
Respiratory	*I felt slightly sore during the video.*
Respiratory	*I felt flu-like symptoms during the video.*

2. Supplementary Results

2.1 Evaluation of video content

The disease and control video were rated with regard to (a) their disgust potential (*"How strong was your feeling of disgust, antipathy and revulsion while watching the video?"*; Likert scale from 0="*not at all*" to 8="*completely*") and (b) the associated contagion risk ("*During the video I had the feeling that I could get infected*"; Likert scale from 0="*completely disagree*" to 7="*strongly agree*"). Utilizing a Wilcoxon signed-rank test, we found that the disease video was on average rated as significantly more disgusting ($z=-5.80$, $p<.001$, $\eta^2=.75$) and more contagious ($z=-4.56$, $p<.001$, $\eta^2=.46$) than the control video.

In a more fine-grained rating of disgust we showed participants single screenshots of the situations shown in the video. Although this rating replicated the significant difference between the videos ($z=5.84$, $p<.001$, $\eta^2=.76$), which we already documented for the broader disgust rating that referred to the whole video, there was also some unexpected interindividual variance in the screenshot disgust rating of the control video (see Figure S1), which led us to perform the exploratory analysis in 2.4.1.1

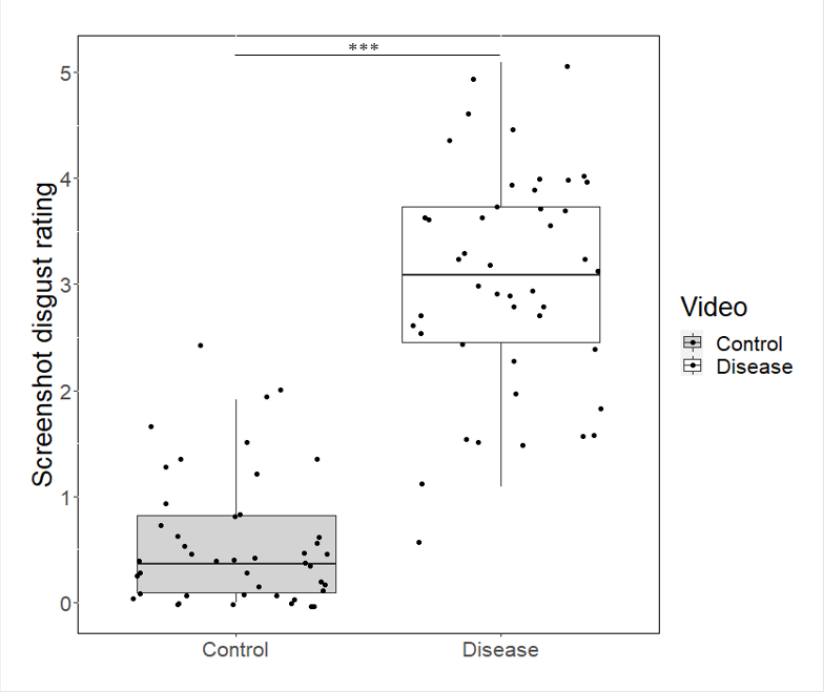

Figure S1: Screenshot disgust rating of control (grey) and disease (white) video. Box represents the median and the 25th and 75th percentiles, whiskers the smallest and the largest value or no further than 1.5*IQR (inter-quartile range). Significant differences are marked with asterisks (***p<.001).

2.2 GLMM Model Coefficients

Table S5: Fixed Coefficient of spike-specific sIgA secretion model with the Fixed Factors *Video* and *Sample* and their Interaction. With Coefficient (β), Standard Error of Coefficient (SE_β), t-value, p-value and lower as well as upper Confidence Intervals (CI).

Model Term	β	SE_β	t	p	95% CI	
					Lower	Upper
Intercept	.05	.18	.29	.773	-.29	.40
Video=Disease	.42	.16	2.58	.010	.10	.74
Video=Control	0[a]					
Sample=Post-Video 2 (PV2)	-.12	.11	-1.06	.291	-.35	.10
Sample=Post-Video 1 (PV1)	-.13	.11	-1.22	.224	-.34	.08
Sample=Baseline	0[a]					
[Video=Disease]*[Sample=PV2]	-.05	.18	-.25	.799	-.41	.31
[Video=Disease]*[Sample=PV1]	.34	.15	2.29	.023	.05	.64
[Video=Disease]*[Sample=Baseline]	0[a]					
[Video=Control]*[Sample=PV2]	0[a]					
[Video=Control]*[Sample=PV1]	0[a]					
[Video=Control]*[Sample=Baseline]	0[a]					

[a]This coefficient is set to zero because it is redundant.

Table S6: Fixed Coefficient of RBD-specific sIgA secretion model with the Fixed Factors *Video* and *Sample* and their Interaction. With Coefficient (β), Standard Error of Coefficient (SE_β), t-value, p-value and lower as well as upper Confidence Intervals (CI).

Model Term	β	SE_β	t	p	95% CI	
					Lower	Upper
Intercept	.87	.09	9.33	<.001	.69	1.05
Video=Disease	.16	.09	1.85	.066	-.01	.33
Video=Control	0[a]					
Sample=Post-Video 2 (PV2)	.35	.15	2.41	.017	.06	.64
Sample=Post-Video 1 (PV1)	.20	.09	2.29	.023	.03	.38
Sample=Baseline	0[a]					
[Video=Disease]*[Sample=PV2]	-.50	.15	-3.33	.001	-.79	-.20
[Video=Disease]*[Sample=PV1]	-.34	.11	-3.16	.002	-.56	-.13
[Video=Disease]*[Sample=Baseline]	0[a]					
[Video=Control]*[Sample=PV2]	0[a]					
[Video=Control]*[Sample=PV1]	0[a]					
[Video=Control]*[Sample=Baseline]	0[a]					

[a]This coefficient is set to zero because it is redundant.

2.3 Confirmatory analysis of total sIgA

In order to confirm that the present disease video had a comparable effect on total sIgA as the disease videos used in our previous study (Keller et al., 2022), we additionally analysed the saliva samples for the content of total sIgA (Please note, in this analysis we had to exclude an additional participant as he was an outlier with regard to the total secretion rate of all 6 saliva samples.). The confirmatory analysis was performed, because the videos from our previous study showed some differences from the presently employed stimulation. The first disease video of our previous only study showed sneezing or coughing people, who often visibly spread aerosols or droplets and never covered their nose or mouth, while the second disease video of that prior study used concealed contagion stimuli such as people lying sick in bed or sneezing into a tissue. Further, both previous disease videos were mute. The presently used disease video showed a mixture of content displaying openly and concealed contagious persons and also contained the sneeze and cough audios for a more realistic stimulation. The control video from our prior study also differed in some important aspects. It primarily showed landscape or street impressions, which seldomly included people, and if so, people were shown only from a

distance and the video never focused on a certain person, whereas the present control video showed healthy people in everyday situations.

In the GLMM with total sIgA we found a significant main effect if *Sample* ($F_{(2,258)}$=13.39, p<.001) as well a significant interaction between *Sample* and *Video* ($F_{(2,258)}$=4.35, p=.014), but no significant main effect of *Video* ($F_{(1,258)}$=1.61, p=.205, please find fixed coefficients in Table S7). In the post-hoc tests, we found a significant difference between the Baseline and the Post-Video 1 sample after watching the disease prime (z=-4.49, p<.001, η^2=.46), as well as after watching the control prime (z=-2.22, p=.027, η^2=.11). However, the increase ($\Delta sIgA_{total}$) after the disease video was significantly higher than the one after the control video (z=-1.75, p=.040, η^2=.07, see Figure S2a). Furthermore, the samples Post-Video 1 and 2 differed significantly after disease video (z=-3.28, p=.001, η^2=.25) but not after the control video (z=-.44, p=.657) (see also Figure S2b).

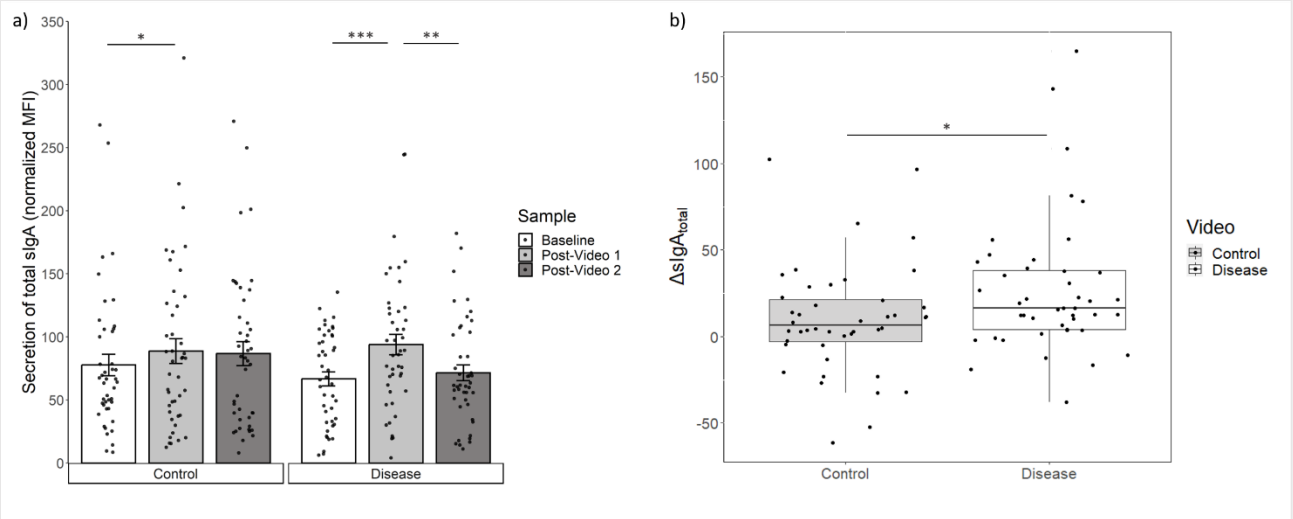

Figure S2: a) Change in total sIgA for disease and control video intervention. Mean, standard errors and individual data points of the secretion rate at Baseline, directly after the video (Post-Video 1), and several minutes after the video (Post-Video 2). Significant changes are marked with asterisks (*p<.05; ***p<.001). b) $\Delta sIgA_{total}$ of the control (grey) and disease (white) video. Box represents the median and the 25th and 75th percentiles, whiskers the smallest and the largest value or no further than 1.5*IQR (inter-quartile range). Significant differences are marked with asterisks (*p<.05).

Table S7: Fixed Coefficient of total sIgA secretion model with the Fixed Factors *Video* and *Sample* and their Interaction. With Coefficient (β), Standard Error of Coefficient (SE_β), t-value, p-value and lower as well as upper Confidence Intervals (CI).

Model Term	β	SE_β	t	p	95% CI	
					Lower	Upper
Intercept	4.18	.10	40.20	<.001	3.98	4.39
Video=Disease	-.10	.11	-.96	.337	-.31	.11
Video=Control	0[a]					
Sample=Post-Video 2 (PV2)	.07	.08	.85	.396	-.09	.22
Sample=Post-Video 1 (PV1)	.08	.06	1.16	.247	-.05	.20
Sample=Baseline	0[a]					
[Video=Disease]*[Sample=PV2]	-.01	.12	-.11	.931	-.24	.22
[Video=Disease]*[Sample=PV1]	.26	.10	2.73	.007	.07	.46
[Video=Disease]*[Sample=Baseline]	0[a]					
[Video=Control]*[Sample=PV2]	0[a]					
[Video=Control]*[Sample=PV1]	0[a]					
[Video=Control]*[Sample=Baseline]	0[a]					

[a]This coefficient is set to zero because it is redundant.

2.4 Explorative analysis of Video Order

2.4.1 Spike-specific sIgA

As an explorative analysis, we ran a second GLMM on spike-specific sIgA with the covariate *Video Order*. This was done, because the first test day, and the type of video one had watched, may have had an influence on spike-specific sIgA secretion on the second test day. We found that the two-way interaction between *Video* and *Sample* was still significant ($F_{(2,258)}$=6.60, p=.002), while the main effects of *Video* ($F_{(1,258)}$=.70, p=.403) and *Sample* ($F_{(2,258)}$=.90, p=.409) were not significant anymore. The additional factor *Video Order* ($F_{(1,258)}$=.23, p=.636) and its two two-way interactions with *Video* Video Order* ($F_{(1,258)}$=.33, p=.565) and *Sample* Video Order* ($F_{(2,258)}$=.701, p=.497) were also not significant. However, the three-way interaction of *Video*Sample* Video Order* ($F_{(2,258)}$=7.33, p<.001) was significant (please find fixed coefficients in Table S9).

When data was split according to Video Order, post-hoc tests on ΔsIgA showed that the increase between Baseline and Post-Video 1 was only significantly higher after the disease video compared to the control video, when participants watched the disease video first (z=-2.71,

p=.007, η^2=.16), but not when they watched the control video on day one (z=1.43, p=.153). Within the participants, who watched the disease video first, we found similar results as in the analysis across the whole group: Accordingly, spike-specific sIgA at Post-Video 1 was significantly higher when having watched the disease video (z=-2.58, p=.010, η^2=.30). There was also a significant difference between Baseline and Post-Video 1 after the disease video (z=-3.00, p=.003, η^2=.41). In participants, who watched the Control video first, we found a significant difference between the Baselines (z=-2.80, p=.005, η^2=.34) and the Post-Video 1 sample (z=-2.01, p=.045, η^2=.18) between the two days. As well as a significant difference between and Post-Video 2 Sample and Baseline (z=-2.46, p=.014, η^2=.26) as well as Post-Video 1 Sample (z=-1.98, p=.048, η^2=.17) on the second day, when participants watched the disease video (also see Tabel S8 and Figure S3).

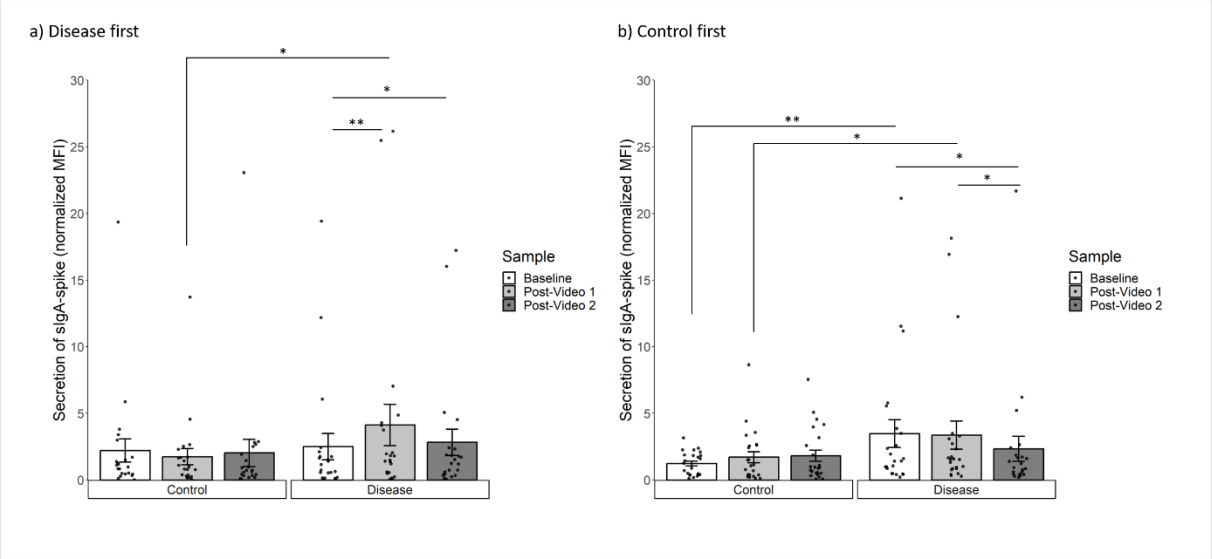

Figure S3: Bar plots with mean, standard errors and individual data points of the spike-specific sIgA secretion rate at Baseline, directly after the video (Post-Video 1), and several minutes after the video (Post-Video 2). For (a) participants (n=22) who watched the disease video first and (b) participants (n=23) who watched the control video first. Significant changes are marked with asterisks (*p<.05; **p<.01), based on Wilcoxon signed rank test.

Table S8: Post-hoc results of Wilcoxon signed rank test wit z values and p-values in parenthesis, comparing spike-specific sIgA at Baseline, Post-Video 1 (PV1) and Post-Video 2 (PV2).

Video Order	**Disease**			**Control**			**Comparison of Videos**		
	Baseline vs. PV1	Baseline vs. PV2	PV1 vs. PV2	Baseline vs. PV1	Baseline vs. PV2	PV1 vs. PV2	Baselines	Post-Video 1	Post-Video 2
Disease first	-3.00 (.003)	-.70 (.485)	-1.74 (.082)	-.63 (.527)	-1.22 (.223)	-.503 (.615)	-.70 (.485)	-2.58 (.010)	-1.64 (.101)
Control first	-.55 (.584)	-2.46 (.014)	-1.98 (.048)	-1.40 (.162)	-1.46 (.144)	-.58 (.563)	-2.80 (.005)	-2.01 (.045)	-.12 (.903)

Table S9: Fixed Coefficient of spike-specific sIgA secretion model with the Fixed Factors *Video*, *Sample* and *Video Order* and their Interaction. With Coefficient (β), Standard Error of Coefficient (SE_β), t-value, p-value and lower as well as upper Confidence Intervals (CI).

Model Term	β	SE_β	t	p	95% CI	
					Lower	Upper
Intercept	.27	.60	.45	.653	-.91	1.45
Video=Disease (D)	-.88	.54	-1.63	.104	-1.94	.18
Video=Control (C)	0[a]					
Sample=Post-Video 2 (PV2)	-.77	.34	-2.26	.025	-1.44	-.10
Sample=Post-Video 1 (PV1)	-.58	.34	-1.68	.094	-1.25	.10
Sample=Baseline	0[a]					
[Video=Disease]*[Sample=PV2]	1.79	.56	3.21	.001	.69	2.89
[Video=Disease]*[Sample=PV1]	1.76	.51	3.48	.001	.77	2.76
[Video=Disease]*[Sample=Baseline]	0[a]					
[Video=Control]*[Sample=PV2]	0[a]					
[Video=Control]*[Sample=PV1]	0[a]					
[Video=Control]*[Sample=Baseline]	0[a]					
Video_order	-.15	.34	-.44	.657	-.83	.53
Video_order*[Video=Disease]	.84	.31	2.74	.007	.24	1.44
Video_order*[Video=Control]	0[a]					
Video_order*[Sample=PV2]	.44	.21	2.05	.041	.02	
Video_order*[Sample=PV1]	.31	.20	1.51	.132	-.09	.70
Video_order*[Sample=Baseline]	0[a]					
Video_order*[Video=D]*[Sample=PV2]	-1.20	.33	-3.62	<.001	-1.86	-.55
Video_order*[Video=D]*[Sample=PV1]	-.92	.28	-3.30	.001	-1.47	-.37
Video_order*[Video=D]*[Sample=Baseline]	0[a]					
Video_order*[Video=C]*[Sample=PV2]	0[a]					
Video_order*[Video=C]*[Sample=PV1]	0[a]					
Video_order*[Video=C]*[Sample=Baseline]	0[a]					

[a]This coefficient is set to zero because it is redundant.

2.4.2 RBD-specific sIgA

When running the same explorative analysis with the covariate of *Video Order* on RBD-specific sIgA, We found that neither the two-way interaction between *Video* and *Sample* was still significant ($F_{(2,258)}$=.48, p=.620), nor the main effect of *Sample* ($F_{(2,258)}$=.90, p=.409) were significant. However, the main effect of *Video* ($F_{(1,258)}$=7.57, p=.001) was now significant. The additional main effect of *Video Order* ($F_{(1,258)}$=.24, p=.623) and its the twoway interaction of *Sample*Video Order* ($F_{(2,258)}$=.27, p=.764) was not significant. The interaction of *Video*Video Order* ($F_{(1,258)}$=13.80, p<.001) was however significant, while the three-way interaction of

*Video*Sample*Video Order* ($F_{(2,258)}$=1.04, p=.356) was not (find fixed coefficients in Table S10).

Table S10: Fixed Coefficient of RBD-specific sIgA secretion model with the Fixed Factors *Video*, *Sample* and *Video Order* and their Interaction. With Coefficient (β), Standard Error of Coefficient (SE_β), t-value, p-value and lower as well as upper Confidence Intervals (CI).

Model Term	β	SE_β	t	p	95% CI	
					Lower	Upper
Intercept	.54	.29	1.85	.066	-.04	1.41
Video=Disease (D)	.63	.31	2.02	.044	.02	1.24
Video=Control (C)	0[a]					
Sample=Post-Video 2 (PV2)	-.12	.52	-.22	.823	-1.13	.90
Sample=Post-Video 1 (PV1)	.12	.27	.46	.646	-.40	.65
Sample=Baseline	0[a]					
[Video=Disease]*[Sample=PV2]	.07	.48	.15	.884	-.88	1.02
[Video=Disease]*[Sample=PV1]	-.29	.37	-.77	.441	-1.03	.45
[Video=Disease]*[Sample=Baseline]	0[a]					
[Video=Control]*[Sample=PV2]	0[a]					
[Video=Control]*[Sample=PV1]	0[a]					
[Video=Control]*[Sample=Baseline]	0[a]					
Video_order	.22	.18	1.20	.233	-.14	.58
Video_order*[Video=Disease]	-.31	.17	-1.85	.065	-.64	.02
Video_order*[Video=Control]	0[a]					
Video_order*[Sample=PV2]	.31	.29	1.06	.290	-.27	.89
Video_order*[Sample=PV1]	.05	.18	.30	.763	-.29	.40
Video_order*[Sample=Baseline]	0[a]					
Video_order*[Video=D]*[Sample=PV2]	-.37	.29	-1.28	.202	-.95	.20
Video_order*[Video=D]*[Sample=PV1]	-.04	.22	-.16	.870	-.47	.39
Video_order*[Video=D]*[Sample=Baseline]	0[a]					
Video_order*[Video=C]*[Sample=PV2]	0[a]					
Video_order*[Video=C]*[Sample=PV1]	0[a]					
Video_order*[Video=C]*[Sample=Baseline]	0[a]					

[a]This coefficient is set to zero because it is redundant.

2.4.3 Total sIgA

Lastly we ran this explorative analysis with the covariate of *Video Order* on total sIgA, we found that the two-way interaction between *Video* and *Sample* ($F_{(2,252)}$=5.04, p=.005) and the main effect of *Sample* ($F_{(2,252)}$=5.41, p=.007) were still significant. The main effect *Video* ($F_{(1,252)}$=.126, p=.722) was still not significant. The additional factor *Video Order* ($F_{(1,252)}$<.01,

p=.961) and the two-way interactions of *Video*Video Order* ($F_{(1,252)}$<.01, p=.970) and *Sample*Video Order* ($F_{(2,252)}$=1.712, p=.183) were also not significant. However, the three-way interaction of *Video*Sample*Video Order* ($F_{(2,252)}$=5.00, p=.007) was significant (find fixed coefficients in Table S11). When data was split by *Video Order*, the post-hoc test on ΔsIgA showed that the increase between Baseline and Post-Video 1 was only significantly higher after the disease video compared to the control video, when participants watched the disease video first (z=-2.19, p=.028, η^2=.12), but not when they saw the control video first (z=-.21, p=.833).

Table S11: Fixed Coefficient of total sIgA secretion model with the fixed factors *Video*, *Sample* and *Video Order* and their Interaction. With Coefficient (β), Standard Error of Coefficient (SE_β), t-value, p-value and lower as well as upper Confidence Intervals (CI).

Model Term	β	SE_β	t	p	95% CI	
					Lower	Upper
Intercept	4.39	.31	13.95	<.001	3.77	5.01
Video=Disease (D)	-.92	.34	-2.72	.007	-1.58	-.25
Video=Control (C)	0[a]					
Sample=Post-Video 2 (PV2)	-.30	.25	-1.22	.223	-.78	.18
Sample=Post-Video 1 (PV1)	-.10	.21	-.48	.631	-.51	.31
Sample=Baseline	0[a]					
[Video=Disease]*[Sample=PV2]	1.06	.35	3.04	.003	.37	1.74
[Video=Disease]*[Sample=PV1]	.98	.31	3.15	.002	.37	1.59
[Video=Disease]*[Sample=Baseline]	0[a]					
[Video=Control]*[Sample=PV2]	0[a]					
[Video=Control]*[Sample=PV1]	0[a]					
[Video=Control]*[Sample=Baseline]	0[a]					
Video_order	-.14	.21	-.66	.511	-.55	.27
Video_order*[Video=Disease]	.54	.20	2.74	.007	.15	.93
Video_order*[Video=Control]	0[a]					
Video_order*[Sample=PV2]	.25	.15	1.67	.096	-.04	.54
Video_order*[Sample=PV1]	.12	.13	.96	.339	-.13	.37
Video_order*[Sample=Baseline]	0[a]					
Video_order*[Video=D]*[Sample=PV2]	-.71	.21	-3.40	.001	-1.13	-.30
Video_order*[Video=D]*[Sample=PV1]	-.47	.18	-2.63	.009	-.83	-.12
Video_order*[Video=D]*[Sample=Baseline]	0[a]					
Video_order*[Video=C]*[Sample=PV2]	0[a]					
Video_order*[Video=C]*[Sample=PV1]	0[a]					
Video_order*[Video=C]*[Sample=Baseline]	0[a]					

[a]This coefficient is set to zero because it is redundant.

2.4.4 Screenshot disgust rating

After having observed the influence of video order on the spike-specific sIgA secretion we decided to run an additional exploratory analysis on the influence of video order on the screenshot disgust rating, to further understand the nature of the potential interpretational bias. We analyzed the screenshot rating after the control the video as well as the rating after the disease video utilizing a Mann-Whitney-U test and found that participants, who watched the control video first, rated it as significantly more disgusting than participants, that watched the disease video first (U=3.35, p<.001, , η^2=.71). This difference, although not significant, was also observed in the rating of the disease video (U=1.80, p=.071, η^2=.72, see Fig. S5). Overall, these results suggest, that watching the control video first lead to a higher disgust rating on both days.

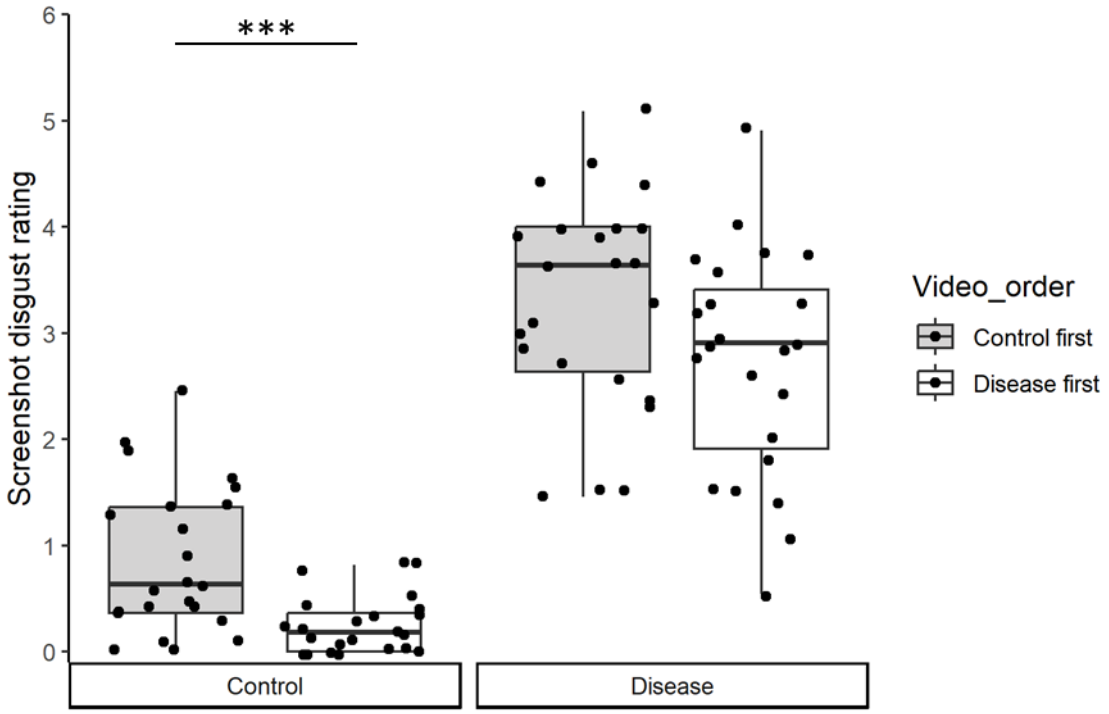

Figure S4: Screenshot disgust rating for the control (left) and disease (right) video of participants that watched the control video first (grey) or disease video first (white). Box represents the median and the 25th and 75th percentiles, whiskers the smallest and the largest value or no further than 1.5*IQR (inter-quartile range). Significant differences are marked with asterisks (***p<.001).

2.5 Difference in interoception scores after the disease video

In order to get a better understanding of the interoception during the disease video we utilized a Wilcoxon-signed-rank test to compare the two composite scores. We found a significant difference between the two interoceptive composite scores related to the disease video. Participants had stronger interoceptive feelings related to the respiratory tract, than gut-related interoceptive feelings ($z=2.78$, $p=.003$, $\eta^2=0.20$) (see Figure S5).

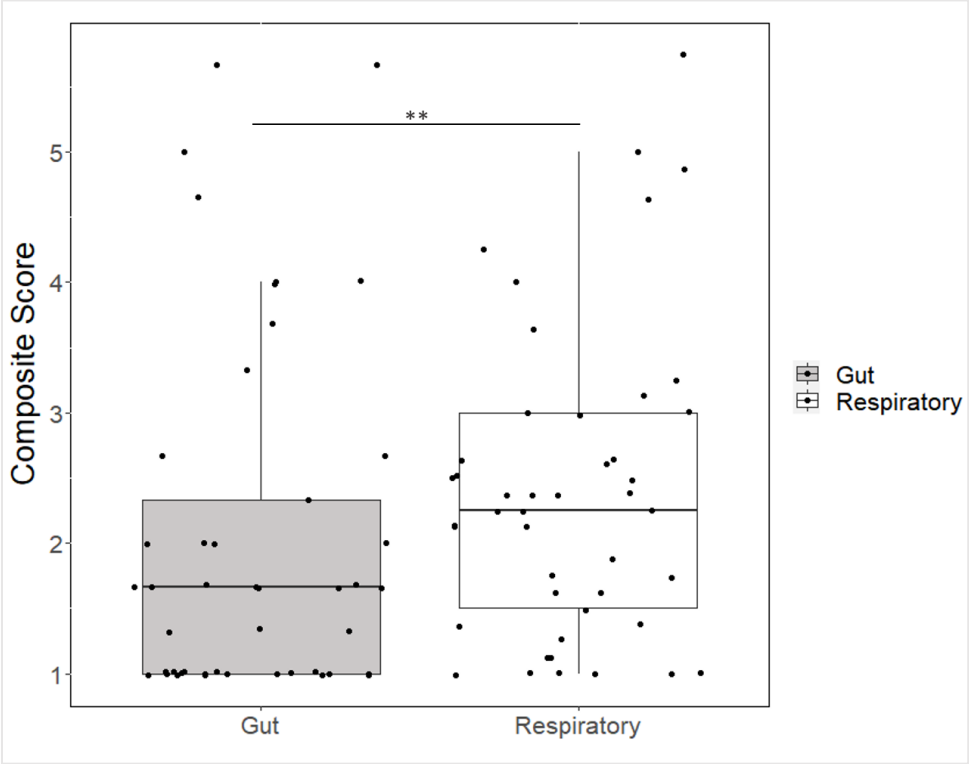

Figure S5: Evaluation of interoceptive feelings after the disease video, regarding the gut (grey) and respiratory (white) composite scores. Box represents the median and the 25th and 75th percentiles, whiskers the smallest and the largest value or no further than 1.5*IQR (inter-quartile range). Significant difference is marked with an asterisk (**p<.01).

2.6 Preregistered GLM-Analysis

Previous to data analysis we preregistered our study with the General Linear Models (GLMs) as primary analysis. After the review process, we decided to switch to GLMM, which may be more appropriate. To keep up transparency of the analyses process, we have added this original analysis here.

2.6.1 SARS-CoV-2-specific sIgA increase after disease-related stimulation

2.6.1.1 Spike-specific sIgA

In order to assess whether the disease video led to an increase in spike-specific sIgA, we performed a 2 x 3 general linear model (GLM) with the factors *Video* (disease, control) and *Sample* (Baseline, Post-Video 1, Post-Video 2). We found a significant main effect of *Video* ($F_{(1,44)}$=4.73, p=.035, ηp^2=.10) and *Sample* ($F_{(2,88)}$=3.33, p=.040, ηp^2=.07), as well as a significant interaction between the two factors ($F_{(2,88)}$=3.71, p=.035, ηp^2=.08). In the post-hoc tests, this was reflected by a significant rise in spike-specific sIgA in the sample directly collected after watching the disease video (Post-Video 1) relative to Baseline (z=-1.80, p=.036, η^2=.72), but not in the corresponding sample taken after the control video (z=-.46, p=.648). Additionally, spike-specific sIgA significantly declined from Post-Video 1 to Post-Video 2 after watching the disease video (z=-2.56, p=.011, η^2=.15), but not after the control video (z=-.12, p=.906). Finally, we found that the samples collected at Post-Video 1 differed significantly between the two videos (disease > control: z=-3.22, p<.001, η^2=.23), while the Baseline (z=-1.59, p=.113) and Post-Video 2 samples (z=-1.25, p=.212) did not (Fig. 1b).

2.6.1.1.1 Exploratory analysis of spike-specific sIgA

Nine of our participants indicated that they were slightly disgusted by the control video. This was unexpected, as the video only displayed healthy people in everyday situations. Since an enhanced feeling of disgust in the control setting could have influenced post-video spike-

specific sIgA, we excluded these participants, who rated more than two of the screenshots from the control video with a score of 4 or higher (this coincided with participants who had an average score above one in the screenshot rating). This left an exploratory sample of 36 participants. In the respective 2 x 3 GLM we found no significant main effect of *Video* ($F_{(1,35)}$=3.81, p=.059), or *Sample* ($F_{(2,70)}$=2.91, p=.061), but replicated the interaction from the total sample ($F_{(2,70)}$=4,34, p=.026, ηp^2=.110).

In the post-hoc tests, we found a significant difference between Baseline and the Post-Video 1 sample after watching the disease video (z=-1.98, p=.024, η^2=.11), but not after watching the control video (z=-.24, p=.648). This was also the case for the difference between the samples taken at Post-Video 1 and Post-Video 2 (Disease: z=-2.75, p=.006, η^2=.21; Control: z=-.58, p=.561). Furthermore, we found that the sample collected at Post-Video 1 differed significantly between the videos (z=-3.39, p<.001, η^2=.32), while the Baseline (z=-1.07, p=.285) and Post-Video 2 sample (z=-1.30, p=.192) did not (see Figure S5a). Finally, the comparison of the rise in spike-specific sIgA from Baseline to Post-Video 1 ($\Delta sIgA_{spike}$) showed that the $\Delta sIgA_{spike}$ of the disease video was significantly higher than the one from the control video (z=-1.95, p=.026, η^2=.11) (see Figure S5b).

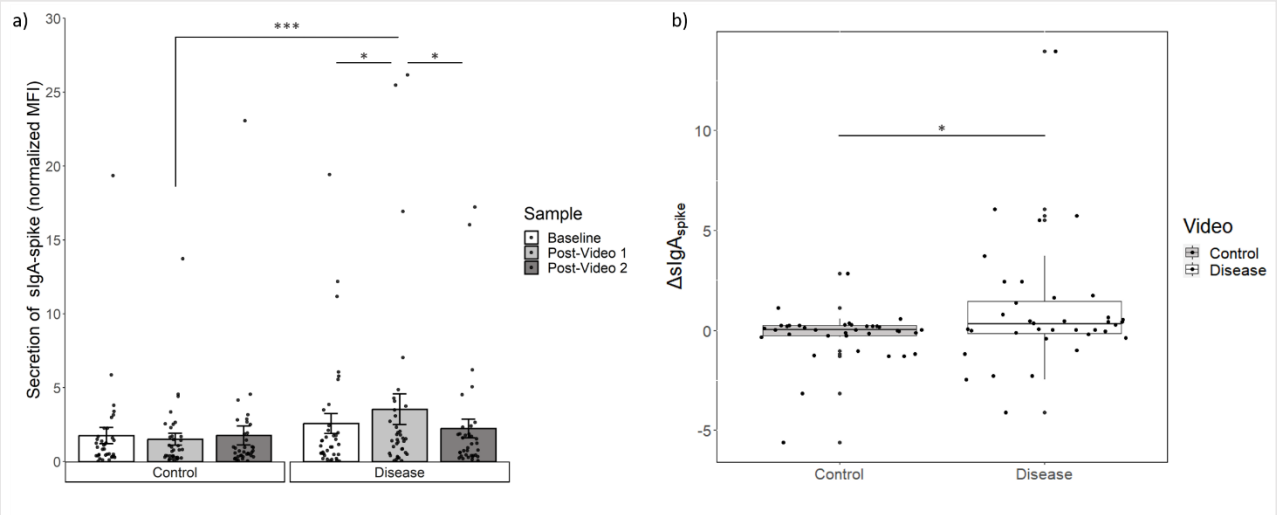

Figure S5: a) Exploratory analysis of spike-specific sIgA in a subgroup of 36 participants. Mean and standard errors of the secretion rate at Baseline, directly after the video (Post-Video 1), and several minutes after the video (Post-Video 2). Significant changes are marked with asterisks (*p<.05; ***p<.001). b) Exploratory analysis of spike-specific ΔsIgA in a subgroup of 36 participants. $\Delta sIgA_{spike}$ of control (grey) and disease (white) video. Box represents the median and the 25th and 75th percentiles, whiskers the smallest and the largest value or no further than 1.5*IQR (inter-quartile range). Significant difference is marked with an asterisk (*p<.05).

2.6.1.1.2 Confirmatory analysis with total sIgA

Finally, we ran a confirmatory analysis of total sIgA, which had significantly increased in response to disease-related video content in our previous study[16]. In the 2 x 3 GLM of total sIgA we found no significant main effect of *Video* ($F_{(1,43)}$=.17, p=.680), but the main effect of *Sample* was significant ($F_{(2,86)}$=10.62, p<.001, ηp^2=.190), as well as the interaction between the factors ($F_{(2,86)}$=6.31, p=.003, ηp^2=.128). In the post-hoc tests, we found a significant difference between the Baseline and the Post-Video 1 sample after watching the disease prime (z=-4.49, p<.001, η^2=.46), as well as after watching the control prime (z=-2.22, p=.027, η^2=.11). However, the increase ($\Delta sIgA_{total}$) after the disease video was significantly higher than the one after the control video (z=-1.75, p=.040, η^2=.07, see Figure S2a). Furthermore, the samples Post-Video 1 and 2 differed significantly after disease video (z=-3.28, p=.001, η^2=.25) but not after the control video (z=-.44, p=.657) (see also Figure S2b).

The $\Delta sIgA_{total}$ was further positively correlated with $\Delta sIgA_{spike}$ for the disease video (rho=.593, p<.001).

2.6.1.2 RBD-specific sIgA

In a second step, we analyzed the RBD-specific sIgA for changes induced by the disease video. In the 2 x 3 GLM we neither found a significant main effect of *Video* ($F_{(1,44)}$=3.03, p=.089, ηp^2=.064) nor of *Sample* ($F_{(2,88)}$=1.12, p=.331, ηp^2=.025), but there was a significant interaction between the two factors ($F_{(2,88)}$=7.60, p<.001, ηp^2=.147). Different from the spike-specific sIgA,

the RBD-specific sIgA showed no significant rise from Baseline to directly after the disease video ($\Delta sIgA_{RBD}$: z=-.37, p=.714), and – similar to the spike-specific sIgA – also not after the control video ($\Delta sIgA_{RBD}$: z=-.04, p=.968). Instead, we found a trend-wise decline in the RBD-specific sIgA from Post-Video 1 to Post-Video 2 (z=-1,95, p=.052, η^2=.08), and also from Baseline to Post-Video 2 (z=-2.18, p=.029, η^2=.11) following the disease video. This indicated a continuous decrease in RBD-specific sIgA throughout the experimental session with the disease video. After the control video, we found a significant increase between Post-Video 1 and Post-Video 2 (z=-2.07, p=.038, η^2=.10), and also when comparing Baseline and Post-Video 2 (z=-2.18, p=.029, η^2=.11) (see Fig. 1c).

3. Supplementary References

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2.3 Chapter III: Influence of female sex hormones on proactive behavioral and physiological immune parameters

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Influence of female sex hormones on proactive behavioral and physiological immune parameters

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ABSTRACT

Women may be more susceptible to infections in the luteal phase, supposedly as a consequence of the hormone progesterone and its immunosuppressive action. While immunosuppression may be important for successful oocyte implantation and pregnancy, it makes women more vulnerable to pathogens. According to theory, to compensate for reduced immunocompetence, women in the luteal phase exhibit proactive behavioral responses, such as disgust and avoidance of disease-associated stimuli, to minimize contagion risk. However, previous studies yielded inconsistent results, and did not account for accompanying proactive immune responses, like the increase of secretory immunoglobin A (sIgA). Here, we assessed the proactive immune response and feelings of disgust associated with disease cues in the comparison of 61 woman with a natural menstrual cycle (31 in the follicular and 30 in the luteal phase) and 20 women taking hormonal contraception (HC). Women rated disease vulnerability and disgust propensity, watched a video displaying people with respiratory symptoms, which was evaluated for its disgust-evoking potential and contagiousness, and provided saliva samples for hormone and sIgA analysis. Women with HC reported a heightened vulnerability to disease compared to naturally cycling women, whereas both the feeling of disgust and the sIgA increase elicited by the disease video were similar across groups, regardless of progesterone. We found a u-shaped relationship between progesterone and baseline sIgA in naturally cycling women, with its nadir during ovulation. Overall, our data do not support a compensatory relationship between the proposed progesterone-induced immunosuppression and heightened disgust or a proactive sIgA response.

1. Introduction

The physiological immune system (PIS) is influenced by various endogenous sex steroid hormones [20]. This is not only reflected by sex differences in the human immune system [21,35], for example the immunosuppressive action of testosterone may render men more vulnerability for communicable diseases than women [31], but also by intra-individual differences driven by hormonal fluctuations that occur during the course of the menstrual cycle [24,43,60]. The menstrual cycle is characterized by a constant change in the concentrations of estradiol and progesterone, among other hormones, which may also affect their relative contribution in immunomodulatory processes. A normal menstrual cycle can be separated into two phases: the pre-ovulatory, follicular phase (marked by low and relatively constant levels of progesterone and rising estradiol, which reaches its cyclic peak right before ovulation), and the post-ovulatory, luteal phase (marked by the highest levels of progesterone during the mid-luteal phase and a second, less pronounced increase in estradiol) [37]. In the luteal phase, women seem to be more liable to infections [43], which has been attributed to the dominating sex hormone of this phase, namely progesterone, which is generally seen as an immune suppressant [23,36,59]. This latter inference has been mainly derived from the role of progesterone in pregnancy and the adaptive advantage of increased progesterone for the successful implantation and subsequent survival of the fertilized embryo [58]. Its crucial participation in the initiation and preservation of pregnancy [48] starts in the luteal phase, i.e., the cycle phase, in which an embryo might successfully implant. It is therefore crucial that the immune system does not reject the blastocyst, which consists of 50% of alien material [14]. While this reduced immune response during the luteal phase is important, it also carries certain risks, as woman become more vulnerable to pathogens and infection. In line with this, the Compensatory Prophylaxis Hypothesis (CPH) [11,12]

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proposes that proactive behavioral responses, like disgust or active avoidance of disease-associated cues, should be upregulated in the luteal phase of woman (and early pregnancy), to compensate for the lack of immunocompetence. While some studies have found support for the CPH [14] (n = 79) [39]; (n = 93) [62]; (n = 30)), others could not find a relationship between cycle phase and/or progesterone level and self-reported pathogen disgust [27] (n = 375) [38]; (n = 527) [41]; (n = 73) [50]; (n = 257)). All these studies used self-report measures for the assessment of pathogen aversion and disgust, while none measured behavioral avoidance and physiological immune responses at the same time and within the same person. Further, studies differed significantly in the cycle phase assignment method (e.g., used methods such as backward-counting and/or forward-counting, partly in combination with hormone levels). However, disgust and avoidance behavior may not be the only proactive immune responses to disease and pathogen cues. Physiological immune responses have been observed to take place way before individuals come into contact with pathogens (i.e., proactive immune responses). Accordingly, visual perception of disgust eliciting items increased Tumor Necrosis Factor - alpha (TNF-α) and Albumin in saliva [53,52] and perception of symptomatic individuals led to an increase in interleukin 6 (IL-6) in blood [47] and secretory Immunoglobulin A (sIgA) in saliva [5,29]. As the main mucosal immunoglobulin, sIgA plays an important role in the first-line-immune-defense against pathogens that enter the body through mouth or nose [61]. There, it is part of several immunological processes such as immune exclusion, i.e., the binding of antigens and prevention of attachment to epithelia cells, and intracellular neutralization, i.e., the neutralization of viral replication in epithelial cells, which play an important role in infection immunity [54]. Its constant secretion into saliva can be rapidly upregulated by (para-)sympathical [6] and mechanical stimulation [44]. In a previous study [29] on the proactive sIgA response to disease cues (i.e., a disease-primer video of sneezing or otherwise ill people) we found evidence for a compensatory relationship between self-reported contamination disgust and the sIgA increase to said cues. However, in that particular study we only tested women that took hormonal contraceptives to control for the potential influence of hormonal fluctuations that occur over the course of the menstrual cycle. Whether sIgA in saliva and its function in immune exclusion in the respiratory tract is influenced by progesterone still remains elusive. There is evidence, that the polymeric immunoglobulin receptor (pIgR), which is a mediator for the transport of IgA across mucosal epithelial cells [28], may be downregulated by progesterone, at least in the endometrium [2]. Two studies that investigated sIgA in saliva during the menstrual cycle had a small sample size (n ≤ 10). While the first study found higher sIgA during the follicular phase [19], the second did not observe a difference between the follicular and luteal phase [18]. A third study with a bigger sample (n = 65) also found no significant difference between cycle phases in oral sIgA [30]. Lastly, a fourth study investigated sIgA during pregnancy, which is associated with even higher progesterone than the luteal phase. It found increased sIgA in 22 pregnant compared to 22 non-pregnant women [46].

In the present study we tested 61 woman that did not use hormonal contraception as well as 20 woman who did. To investigate if their proactive physiological immune response to disease cues as well as behavioral mechanisms of pathogen avoidance were influenced by variations in sex hormones, we exposed the women to a disease-primer video showing humans with typical respiratory symptoms, similar to the video used in the previous study [29]. We measured salivary sIgA (before and after the video), the morning titer of progesterone and estradiol, as well as self-reported disgust and contagion risk. We expected, that the proposed immunosuppressive effect of heightened progesterone during the luteal phase would have an attenuating effect on the proactive sIgA response (i.e., lead to a smaller or no increase) to the disease-primer video. Based on the CPH, we further predicted that high progesterone should be accompanied by other proactive behavioral mechanisms (e.g., increased self-reported disgust) that compensate for the reduced physiological immunocompetence.

2. Methods

2.1. Participants

We tested three groups of biological women in a between-subjects design. One group was tested during the intake-phase of hormonal contraception (HC) (day 2–21 of HC), while the other two groups were tested once during their menstrual cycle, in either the follicular or the luteal phase. The respective cycle phase was determined based on the criteria described below. We performed a power analysis with G*power [10], and used the '*F-test: ANOVA with repeated measures, and within-between interaction*' that included three groups (follicular phase, luteal phase, HC) and four measurements (four saliva samples). For a power of (1-β) = .90 to detect a medium effect of f = .20 with α = .05 (correlation among repeated measures = .50, nonsphericity correction = 1) the analysis indicated a sample size of 57 women. To account for potential drop-out and non-compliance during the remote test, we recruited as many women as possible on the campus of the University of Hamburg (Germany), through online advertisements and via social media, which resulted in 89 women in total. We only invited healthy individuals to participate, who (a) indicated German as their native language, (b) were of legal age but not older than 35 years, (c) were not smoking regularly, (d) had no hormonal, genetic, or other chronic diseases, (e) reported a regular cycle between 25 & 38 days (f) had not been vaccinated in the last 2 weeks, and (g) were willing to participate online for the approximate duration of 1 h. Women received a financial reward of 20 Euros for completing the test appointment. We obtained informed consent from all women and the procedure was approved by the local ethics committee *"Ethikkommission der Ärztekammer Hamburg"* and conformed with the Declaration of Helsinki.

2.2. Procedure

On the test day, women gave three saliva samples in 30 min increments right after waking up. These morning samples were later pooled and used to determine the morning estradiol and progesterone level in saliva. For the actual test session women than joined a Zoom call with the experimenter between 1 and 5 pm. At the beginning of the test session, women collected the first saliva sample (baseline sample). Afterwards, they were transferred to an online survey that was programmed with the software Limesurvey. There, they provided demographic data on aspects such as age, gender, current state of health (*'very sick'*, *'healthy'*, and *'very fit'*) and general everyday stressors (e.g., smoking, sports, alcohol, current and previous diseases, etc.). In the following, women evaluated their disgust sensitivity on the modified version of the Disgust Scale established by Haidt et al. [22] and revised by Olatunji et al. ([42], DS-R), translated into German. This scale consisted of 17 items (α = .74), eight of these were true-false items with statements like *'I might be willing to try eating monkey meat, under some circumstances.'*, for which women indicated their agreement on a 5-point Likert-scale (from 0 = '*Strongly disagree*' to 4 = *'Strongly agree'*). The rest of the items had the participant rate situations for their disgust-eliciting potential. For example: *'While you are walking through a tunnel under a railroad track, you smell urine.'* (from 0 = *'Not disgusting at all'* to 4 = *'Extremely disgusting'*).

After finishing the survey, the women were transferred to the online platform testable.org, were they watched the disease-primer video. This video was intended to trigger fear of contagion by a respiratory disease. It comprised video clips and pictures of people, who were sneezing, or showed other common signs of respiratory viral infections, e.g., looked feverishly or lay sick in bed. The sneezes were either concealed by a tissue, hand or arm, or were unconcealed, i.e., the displayed persons sneezed directly into the camera or in the vicinity, whereby some individuals visibly emitted aerosols or mucus. The short videos clips and

pictures were assembled to a disease-primer video of 05:00 min. It was repeated once to achieve a video stimulation of 10 min. The video clips and pictures were acquired from platforms such as pexels.com, pixaby.com and istockphoto.com. The majority of the openly sneezing people were from the *'Bless-you'* video by Ulf Lundin. The disease-primer video was followed immediately by the second saliva sample (i.e., the Post-Video 1 sample). The complete stimulation video is not publicly accessible due to copy right restrictions.

After the disease-primer video, women were asked to recall the feelings they experienced while watching the video. For this 'absolute recall task', we asked them how they had felt, while watching the video using 6 statements, such as '*How strong was your feeling of disgust, antipathy and revulsion while watching the video?*' (from now on referred to as 'Disgust-rating'). The statements about the feelings had to be rated on a 9-point Likert-scale from 0 *'not at all'* to 8 *'completely'* [4,16].

This was followed by questions about the video content. We first asked three questions that required a recall of details, such as '*How many elderly men were portrayed in the video?*'. The women had a choice between five options, such as '*None*', or '*Only 1 elderly man*'. Furthermore, women were shown 15 pictures of which 10 were screenshots from the video previously shown, while 5 had not been shown in the video. The women were asked, '*Was this person/situation portrayed in the video?*' and had to choose between the options '*Yes*' and '*No*'. These questions allowed us to implicitly evaluate whether the women had payed attention to the details of the video. Women that answered less than 50% of the questions correct would have been excluded, but all passed the attention check. Afterwards women were asked to evaluate how ill and contagious each of the persons shown in the disease-primer video was. For this, we presented screenshots from the video and asked to rate the statement *'I would catch an infection from the person shown'* on a scale from 1 *'Very unlikely'* to 4 *'Very likely'* (from now on referred to as 'Contagion-Rating' (26 items, $\alpha = .93$) as well as the question *'How ill was this person at the time of recording?'* on a scale from 1 *'not ill at all'* to 4 *'severely ill'* (from now on 'Illness-Rating' (26 items, $\alpha = .91$)). These ratings were than followed by the third saliva sample (Post-Video 2 sample). We ensured, that at least 10 min passed between the beginning of this sample and the beginning of the one before that (Post-Video 1). We further asked the women more general personality questions, which were not disease- or disgust-associated, and were not relevant to the current study, but insured that another 10 min passed before the fourth and last saliva sample was collected (Post-Video 3 sample).

Finally, women evaluated their perceived Vulnerability to Disease (pVtD), by using a 15-item self-report instrument designed by [8] and translated into German. These 15 items ($\alpha = .75$) included statements like *'In general, I am very susceptible to colds, flu and other infectious diseases.'* and *'I prefer to wash my hands pretty soon after shaking someone's hand.'*, which the women had to evaluate on a 7-points scale (from 1 *'strongly disagree'* to 7 *'strongly agree'*).

2.3. Saliva samples

The women received a kit for saliva collection at home. It was sent by mail, since the restrictions related to the COVID-19-pandemic precluded tests in our computer lab at the university. The kit included seven 2 mL microcentrifuge *eppendorf* tubes and an instruction of proper saliva sample collection (see Supplement; Instructions for saliva sampling 1). Once the experiment was completed, the envelope with the samples was sent back to the institute on the same day via *Deutsche Post Versand*, which took 1–2 days. In the lab, samples were immediately frozen at −20 °C.

For analysis of steroid hormones, women gave three saliva samples in 30 min increments right after waking up on the day of testing. Equal amounts of supernatants were collected from the thawed and centrifuged samples and were then pooled and sent on dry ice to *ISD Laboratory,* Malente, Germany. There, hormone concentrations (pg/mL) were determined using DRG Salivary Progesterone ELISA (SLV-2931, with a sensitivity of 3.8 pg/mL) and DRG Salivary Estradiol ELISA (SLV-4188, with a sensitivity of 0.6 pg/mL). All samples were analyzed in duplicates and had a mean CV of 5.13% (SD = 4.96%) for estradiol and 5.78% (SD = 5.39%) for progesterone.

The four saliva samples collected during the test session (i.e., baseline, Post-Video 1–4 samples), were sent on dry ice to the *MVZ Laboratory Volkmann*, Karlsruhe, Germany. There, an immuno-nephelometric analysis was completed on the Atellica® NEPH 630 System to determine the concentration of sIgA (mg/dL) in saliva. The sIgA raw values from this analysis were than individually calculated with the time it took each woman to provide the saliva sample (mean ± SD = 2.94 min ± 2.23), to get the individual concentration per minute.

2.4. Assignment of cycle phase

Many aspects have to be considered when sorting biological women by cycle phase [24]. In this study, we decided to not only determine cycle phase based on self-reported information related to average cycle length and last menstruation-onset alone, but also measured individual progesterone and estradiol concentration in morning saliva. In addition, we asked women to inform us about the onset of the next menstruation following the test appointment.

To ensure that women were in the right group for data analysis we took two analytical approaches: Firstly, we determined group (follicular or luteal phase) based on a combination of common forward- and backward-counting methods [3,7]. Upon recruitment, the women informed us about (1.) their average cycle length in the last three months, (2.) the onset of the last menstruation, and (3.) the expected start of the next menstruation (expected date of next menstruation). Based on this information, we randomly scheduled an appointment in either the follicular or the luteal phase. After their test appointment, the women then informed us about the actual start of their next menstruation as soon as it started (date of next menstruation). The date of next menstruation allowed us to calculate the actual cycle length of the test cycle, which could be different from the initially predicted cycle length. We then calculated the standardized cycle day (SCD) of the test appointment in the following way: $\left(\frac{\textit{Days since start of last menstruation}}{\textit{Actual cycle length}}\right) * 28$ (see also [7]).. In that way, all data were treated as if all women had an ideal 28-days cycle. The women with a standardized cycle day between 1 and 15 were then sorted into the follicular phase, while women with a standardized cycle day between 16 and 28 were sorted into the luteal phase.

In a second approach, to approximate for potential faults of the counting-method, we utilized a median split method based on salivary progesterone to separate the women into the two groups. The women, who did not take hormonal contraceptives, had a median progesterone value of 36.4 pg/mL. Women below this value were sorted into the follicular phase, while women above it were sorted into the luteal phase. We than excluded the women, who were assigned to different cycle phases by the two calculations, e.g., who belonged to the follicular phase according to the counting-method and the luteal phase according to the progesterone-based median split method (combined method; CM; $n_{excluded} = 15$, see Fig. 1). The hormonal contraceptive group was assigned based on the personal information of the women that they currently used hormonal contraceptives (detailed information on hormonal contraceptives see Supplementary Table 1).

2.5. Data analysis

All analyzes were conducted using IBM SPSS 29, graphs were created with RStudio and MS Excel 2016. Firstly, we tested all variables for deviation from a normal distribution utilizing the Kolmogorov-Smirnoff test with a significance threshold of $p < .050$ (see Supplementary Table 2). We compared the non-parametric hormone levels of the three groups using a Kruskal-Wallis test. Significant results were further

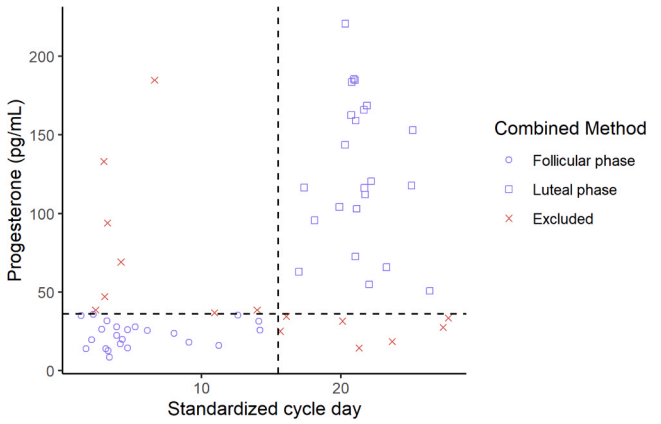

Fig. 1. Assignment of cycle phases based on combined method. Progesterone of all women not taking hormonal contraceptives (n = 61) plotted against standardized cycle days. Horizontal line is equivalent to progesterone median. Vertical line is marking group threshold for standardized cycle day method.

investigated with Mann-Whitney-U- test which were adjusted with the Bonferroni correction (p_b). for a similar procedure also applied to the non-parametrically distributed questionnaire scores (Contamination Disgust Subscale, Core Disgust subscale, Illness-Rating and State disgust) as well as the Baseline sIgA concentration. The difference in the normally distributed questionnaires (All pVtD Scales, Disgust Sum Score and Contagion rating) between the three groups were tested with one-way ANOVAs and post-hoc unpaired t-tests.Lastly, to investigate the interaction between the change in sIgA and the three groups we ran a 4 × 3 repeated measures GLM with *Sample* (Baseline, Post-Video 1, Post-Video 2, Post-Video 3) as within-subject factor and *Group* (follicular phase, luteal phase and hormonal contraception) as between-subjects factor. Post-hoc tests of significant effects were done with Wilcoxon-Signed-Rank tests, and corrected for multiple comparisons with the Bonferroni correction.The data that support the findings of this study are openly available under https://osf.io/yzu95/.

3. Results

Of the 89 originally recruited women, we excluded the data of women who had a vaccination (n = 2) or an infection (n = 1) less than two weeks before testing and failed to inform us beforehand. Further, we excluded women who were outliers (mean ± 2 * SD) in the concentration of morning progesterone (n = 3), estradiol (n = 1) or in all of the sIgA measurements (n = 1). This left a sample of 81 women with a mean age of 25.35 years (SD = 3.65) and with the 61 naturally cycling woman having a mean cycle length of 29.31 days (SD = 3.30). For group sizes in the different assignment methods please see Table 1.

Table 1
Group assignment according to the two sorting methods with n per group as well as average hormonal level in pg/mL (mean ± SD).

		Group assignment		
Assigned based on…		Follicular phase	Luteal phase	Hormonal contraceptives
Standardized cycle day (SCD)	n	31	30	20
	Estradiol	4.26 (± 1.81)	4.27 (± 1.80)	4.91 (± 2.17)
	Progesterone	37.83 (± 37.11)	103.55 (± 59.97)	21.94 (± 8.24)
Combined Method (CM)	n	23	23	20
	Estradiol	3.47 (± 0.72)	4.22 (± 1.75)	4.91 (± 2.17)
	Progesterone	23.04 (± 7.88)	127.00 (± 47.56)	21.94 (± 8.24)

3.1. Hormone levels

First, we compared the hormone levels between the three groups (follicular phase, luteal phase, and HC) and in each of the two assignment methods (counting method, combined method).

We found no significant difference between groups regarding the estradiol concentration in the two assignment methods (SCD: $H_{(2)} = 2.08$, $p = .354$, $\eta^2 = .001$; CM: $H_{(2)} = 5.89$, $p = .053$, $\eta^2 = .06$, see Table 1).

Moreover, progesterone concentration differed between the groups in the two methods (SCD: $H_{(2)} = 32.83$, $p < .001$, $\eta^2 = .40$; CM: $H_{(2)} = 44.44$, $p < .001$, $\eta^2 = .67$). Progesterone was highest in women during the luteal phase, when compared to women in the follicular phase (SCD: $U = -25.26$, $p < .001$, $p_b < .001$, $\eta^2 = .66$; CM: $U = -31.94$, $p < .001$, $p_b < .001$, $\eta^2 = .57$), and women taking hormonal contraceptives (SCD: $U = 36.51$, $p < .001$, $p_b < 001$, $\eta^2 = .55$; CM: $U = 34.23$, $p < .001$, $p_b < .001$, $\eta^2 = .53$). Women in their follicular phase did not differ from the ones taking hormonal contraceptives (SCD: $U = 11.24$, $p = .096$, $p_b = .288$, $\eta^2 = .65$; CM: $U = 2.29$, $p = .696$, $p_b > 1.0$, $\eta^2 = .72$).

3.2. Perceived vulnerability to disease

We found a significant difference between the three groups in the total score of the perceived Vulnerability to Disease questionnaire (SCD: $F_{(2,78)} = 4.6$, $p = .013$, $p\eta^2 = .11$; CM: $F_{(2,63)} = 3.92$, $p = .025$, $p\eta^2 = .11$), with women taking hormonal contraceptives having a significantly higher score than women in the follicular phase (SCD: $t = -2.23$, $p = .031$, $p_b = .093$, $d = -.64$.; CM: $t = -2.53$, $p = .015$, $p_b = .045$, $d = -.77$) and those in the luteal phase (SCD: $t = -3.26$, $p = .002$, $p_b = .006$, $d = -.94$.; CM: $t = -2.61$, $p = .013$, $p_b = .039$, $d = -.80$). There was no significant difference between follicular and luteal phase women (SCD: $t = .79$, $p = .435$, $p_b > 1$, $d = .20$.; CM: $t = -.22$, $p = .827$, $p_b > 1$, $d = -.07$, see Fig. 2a).

Similarly, the Germ Aversion subscale also differed between groups (SCD: $F_{(2,78)} = 4.29$, $p = .017$, $p\eta^2 = .01$; CM: $F_{(2,63)} = 4.02$, $p = .023$, $\eta^2 = .11$), with women taking hormonal contraceptives having a significantly higher score than women in the follicular phase (SCD: $t = -2.28$, $p = .027$, $p_b = .081$, $d = -.654$.; CM: $t = -2.36$, $p = .023$, $p_b = .069$, $d = -.72$) and those in the luteal phase (SCD: $t = -3.24$, $p = .002$, $p_b = .006$, $d = -.936$; CM: $t = -3.00$, $p = .005$, $p_b = .015$, $d = -.92$). There was no significant difference between follicular and luteal phase (SCD: $t = .51$, $p = .607$, $p_b > 1$, $d = .13$; CM: $t = .14$, $p = .890$, $p_b > 1$, $d = .04$, see Fig. 2b).

We found no significant difference in the perceived Infectability subscale (SCD: $F_{(2,78)} = 2.48$ $p = .091$, $p\eta^2 = .06$; CM: $F_{(2,63)} = 2.16$, $p = .124$, $p\eta^2 = .06$. see Fig. 2c).

3.3. Trait disgust

We found no significant difference between the three groups in the Disgust Sum Score from the DS-R (SCD: $F_{(2,62)} = .90$, $p = .410$, $p\eta^2 = .02$; CM: $F_{(2,62)} = .08$, $p = .925$, $p\eta^2 = .003$). This was also the case for the Contamination Disgust subscale (SCD: $H_{(2)} = 2.61$, $p = .271$, $\eta^2 = .008$; CM: $H_{(2)} = .26$, $p = .877$, $\eta^2 = .03$) and Core Disgust subscale (SCD: $H_{(2)} = 2.37$, $p = .255$, $\eta^2 = .005$; CM: $H_{(2)} = .47$, $p = .791$, $\eta^2 = .02$).

3.4. State feelings

Regarding the state feelings following the video we were not able to find any significant differences between the three groups in the Contagion-rating (SCD: $F_{(2,62)} = 1.92$, $p = .153$, $p\eta^2 = .05$; CM: $F_{(2,62)} = 1.47$, $p = .238$, $p\eta^2 = .05$), the Illness-rating (SCD: $H_{(2)} = 1.36$, $p = .507$, $\eta^2 = .005$.; CM: $H_{(2)} = .72$, $p = .698$, $\eta^2 = .02$), and in the Disgust-rating after the video (SCD: $H_{(2)} = 1.59$, $p = .452$, $\eta^2 = .005$; CM: $H_{(2)} = .63$, $p = 0.729$, $\eta^2 = .02$).

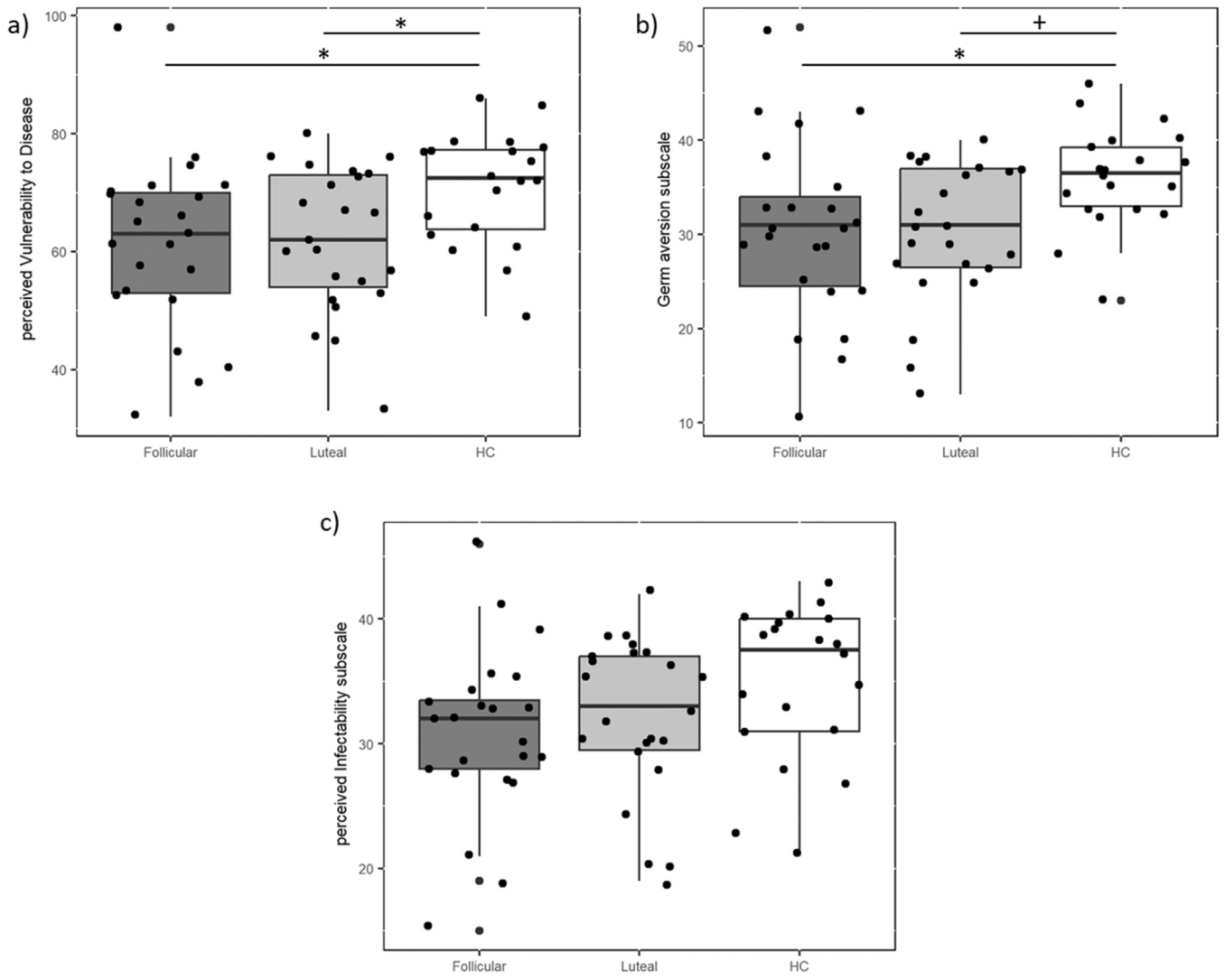

Fig. 2. Perceived vulnerability to disease across groups. Combined Method (CM): Comparison of the three groups a) total score of perceived Vulnerability to Disease questionnaire, b) Germ Aversion subscale, and c) perceived Infectability subscale. Significant differences are marked with asterisks (*$p_b < .050$, $^{+}p_b < .100$).

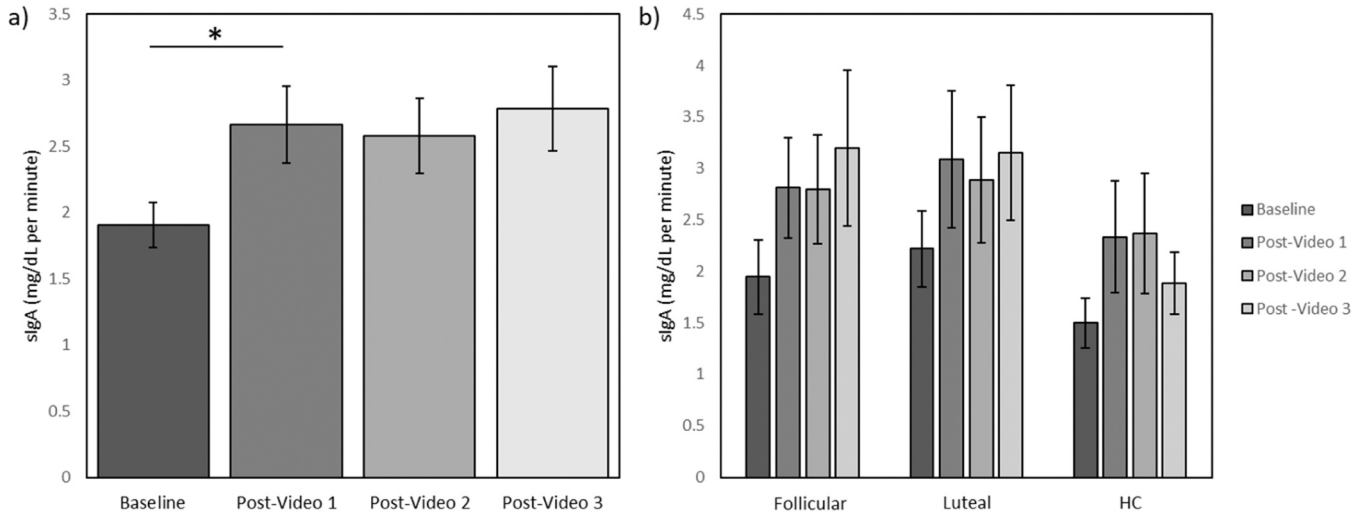

Fig. 3. Change of sIgA during the experiment. a) Change of sIgA across all participants (n = 81). b) Combined method (n = 61): Change of sIgA in each of the three groups. Mean and standard errors of the secretion per minute at Baseline, directly after the video (Post-Video 1), and several minutes after the video (Post-Video 2 & 3). Significant differences are marked with asterisks (*$p < .05$).

3.5. Differences in mucosal immunity

Investigating the differences in mucosal immunity, we firstly compared the sIgA baseline of the three groups. There was no difference between the groups (SCD: $H_{(2)} = .86$, $p = .652$, $\eta^2 = .02$; CM: $H_{(2)} = 1.42$, $p = .491$, $\eta^2 = .01$). Further, in the 4 × 3 *Sample* x *Group* repeated measures GLM we found a significant main effect of *Sample* in both methods of assignment (SCD: $F_{(2.43,189.19)} = 4.26$, $p = .010$, $\eta p^2 = .05$; CM: $F_{(2.44,153.89)} = 3.97$, $p = .014$, $\eta p^2 = .06$), but there was neither a main effect of the factor *Group* (SCD: $F_{(2,78)} = .71$, $p = .493$, $p\eta^2 = .02$; CM: $F_{(2,63)} = .95$, $p = .394$, $p\eta^2 = .03$), nor an interaction of *Sample* × *Group* (SCD: $F_{(4.85,189.19)} = .47$, $p = .792$, $p\eta^2 = .01$; CM: $F_{(4.89,153.89)} = .38$, $p = .859$, $p\eta^2 = .01$, see also Fig. 3b for a similar change in each of the three groups).

We investigated the main effect of *Sample* with post hoc Wilcoxon signed-rank tests. These revealed a significant increase between the Baseline sample and the one taken directly after the video (Post-Video 1) (SCD: $z = -2.83$, $p = .005$, $p_b = .027$, $\eta^2 = .10$, see Fig. 3a; CM: $z = -2.81$, $p = .005$, $p_b = .030$, $\eta^2 = .12$), but no further differences between Baseline and Post-Video 2 (SCD: $z = -2.14$, $p = .032$, $p_b = .0195$, $\eta^2 = .06$; CM: $z = -2.52$, $p = .012$, $p_b = .072$, $\eta^2 = .10$), Baseline and Post-Video 3 (SCD: $z = -2.42$, $p = .015$, $p_b = .093$, $\eta^2 = .07$; CM: $z = -2.35$, $p = .019$, $p_b = .114$, $\eta^2 = .08$), Post-Video 1 and Post-Video 2 (SCD: $z = -.89$, $p = .375$, $p_b > 1$, $\eta^2 = .01$; CM: $z = -.35$, $p = .728$, $p_b > 1$, $\eta^2 = .002$), Post-Video 1 and Post Video 3 (SCD: $z = -.50$, $p = .621$, $p_b > 1$, $\eta^2 = .003$; CM: $z = -.36$, $p = .723$, $p_b > 1$, $\eta^2 = .002$), nor Post-Video 2 and Post-Video 3 (SCD: $z = -.09$, $p = .932$, $p_b > 1$, $\eta^2 = .0$; CM: $z = -.79$, $p = .430$, $p_b > 1$, $\eta^2 = .01$) emerged (for change of sIgA concentration in all three groups see Fig. 3b).

3.6. Explorative analysis: regression

Sex hormones throughout the cycle are not only variable in their concentration, but their effects may also depend on accompanying changes in receptor density and other cellular factors that are only partly understood, which often results in complex non-monotonic dose-response relationships [32,56]. Therefore, we decided to investigate the relationship between the sIgA samples and morning progesterone further, using a regression analysis on the data of women with naturally fluctuating hormone levels that where included in the combined assignment method (n = 46). As data distribution was non-parametric, we performed a rank transformation before fitting the regressions. The data were fitted to a linear and a quadratic function, yet the linear function yielded no significant results (Baseline: $R^2 = .002.$, $F_{(1.44)} = .10$, $p = .756$ (see Fig. 4a); Post-Video 1: $R^2 < .001.$, $F_{(1.44)} = .002$, $p = .965$; $\Delta sIgA_1$:: $R^2 < .001.$, $F_{(1.44)} = .02$, $p = .898$ [$\Delta sIgA_1$ = Post-Video 1 – Baseline]). However, for the quadratic regression we found that morning progesterone predicted sIgA concentration at baseline ($R^2 = .21$, $F_{(2.43)} = 5.61$, $p = .007$; $\beta = 1.94$, $p = .002$, see Fig. 4b), but neither at Post-Video 1 ($R^2 = .03$, $F_{(2,43)} = .63$, $p = .537$), nor in relation to the increase from baseline to Post-Video 1 ($\Delta sIgA_1$) ($R^2 = .03$, $F_{(2,43)} = .55$, $p = .581$).

4. Discussion

In an attempt to get a better understanding of the influence of steroid hormones on proactive immune responses to disease cues, we presented female participants, who were either in the luteal or follicular phase of their cycle or who took hormonal contraceptives, a disease-primer video that displayed people with typical symptoms of respiratory infection (e.g., sneezes, feverish skin). Before video presentation, participants filled out a self-report questionnaire on trait disgust. Directly after the video they then indicated their state feelings related to disease perception and contamination disgust, and finally answered a trait questionnaire on perceived disease vulnerability. In addition, participants provided several saliva samples for sIgA analysis before, during and after the experiment. We found that the woman taking hormonal contraceptives showed an increased self-reported vulnerability to disease relative to women with a natural menstrual cycle. Further, our data showed that natural fluctuations of progesterone may be a non-linear predictor of sIgA at baseline. Yet, we were unable to find any support for the compensatory prophylaxis hypotheses, since neither self-reported feelings of state or trait disgust, nor the proactive physiological immune response of sIgA to the video stimulation differed between the three test groups.

Previous findings on differences in self-reported disgust across the menstrual cycle have been mixed. Our findings are in line with studies reporting no impact of the menstrual cycle on disgust sensitivity [27,38,41,50], yet contradict findings supporting the CPH [14,39,62]. The reasons for previous inconsistencies have been widely discussed by Fleischman and Fessler [15] in response to Jones et al. [27]. Fleischman and Fessler [15] thereby proposed three possible reasons: The first was based on differences in the measurement methods used by the studies. For example, Jones et al. [27] used the trait disgust questionnaire Three Domain Disgust Scale (TDDS, [55]), while Fleischman and Fessler [14] used picture ratings capturing the state disgust triggered by the stimuli. These two methods investigate disgust propensity from different angles. While trait disgust reflects a stable tendency to experience disgust that relies on self-report related to imagined disgust-evoking situations, state disgust represents the actual emotional reaction following exposure to potentially disgust-evoking items [45]. In our study we combined both

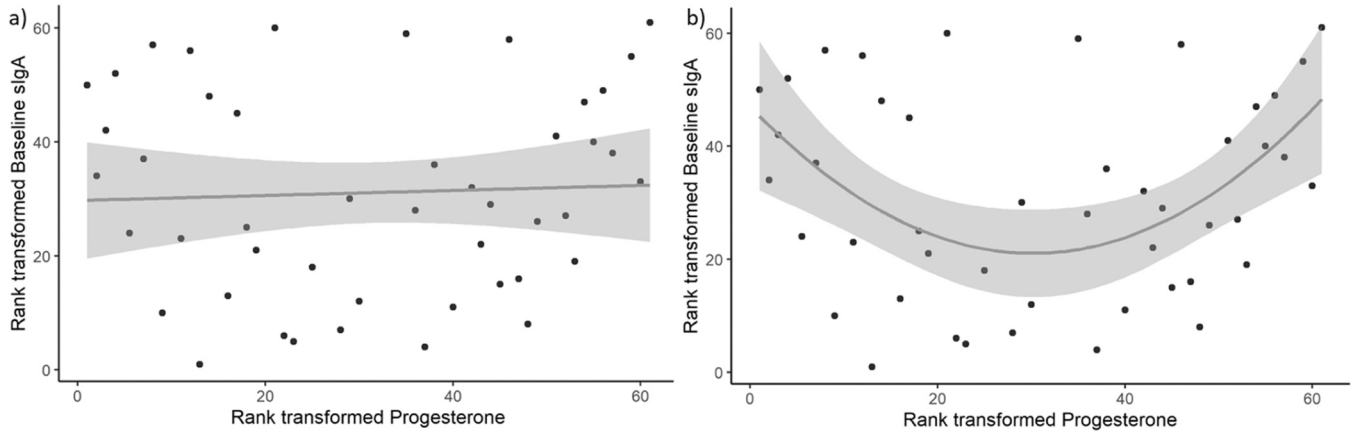

Fig. 4. Relationship between progesterone and Baseline sIgA (both rank transformed). a) Scatter plot with a linear model based on the data with 95% confidence interval in gray and b) Scatter plot with a quadratic model based on the data with 95% confidence interval in gray.

approaches and let participants evaluate trait disgust (DS-R) as well as their state disgust (Stimulus rating). Yet, we found no difference between menstrual cycle phase nor differences between naturally cycling women and those taking hormonal contraceptives. Furthermore, the cycle phase assignment method did not alter the null results and our between-subjects design found similar results as previous within-subject designs [27,50]. Therefore, we suggest that cycle phase assignment methods could be excluded as source of the differing findings regarding the CPH. Another explanation for differing results offered by Fleischman and Fessler [15] was that progesterone is not the actual driving factor of prophylactic behaviors. While our group data also indicate this, since we found no significant effect of the menstrual cycle phase or hormonal contraceptives on the sIgA baseline, the non-linear relationship between progesterone and sIgA in saliva may indicate a possible non-monotonic dose-response relationship between sIgA and progesterone rather than a linear or threshold dependent response [56]. We found that sIgA was lowest when progesterone levels neared the nadir, similar to the progesterone level around ovulation. This decline in sIgA around ovulation has also been found in the cervical mucus [9,33,49]. While Shrier et al. [49] found a negative correlation between sIgA in the cervical mucus and estradiol we could not find a similar correlation in our explorative analysis regarding sIgA in saliva and estradiol (see Supplementary Table 3). Kutteh et al. [33] on the other hand speculate about a dilution affect as cervical mucus is increased during ovulation, however this is not the case for mucus in saliva. Ovulation is a complex process in the female body that is regulated by many factors and is accompanied by a lot of hormonal changes [26], therefore it is impossible to pin point the driver for the sIgA decrease around ovulation without further research. A better understanding of the different drivers and the role of progesterone can only be achieved with further research, including replication studies, with a specific focus on ovulation or studies in pregnant woman as suggested by Stern and Shiramizu [50]. However in the study of Jones et al. [27] neither progesterone showed a relationship with prophylactic behaviors, nor did testosterone, estradiol or cortisol. Therefore, the third and last possible explanation of Fleischman and Fessler [15] needs to be considered as well: the CPH may be wrong.

We are the first to consider both self-reported disgust as a proactive prophylactic mechanism and the upregulation of physiological immune parameters following disease-associated visual stimulation. In a previous study [29] researchers found that videos of people displaying respiratory disease symptoms triggered a proactive increase of sIgA in saliva (way before pathogen exposure), and this proactive increase inversely correlated with the contamination subscale of the DS-R. This was interpreted as a compensating relationship between a physiological and a behavioral proactive immune mechanism that each reduces contagion risk. However, the previous study found no relation between disgust and baseline physiological immune responses. In the current study, we were able to replicate the proactive increase of sIgA in saliva after disease-related visual stimulation with a medium effect, comparable to the one of Keller et al. [29]. This increase, however, neither depended on the menstrual cycle phase nor on the intake of hormonal contraceptives, and thus may not be affected by group differences in steroid hormones. This is also supported by the results of an exploratory regression analysis, which revealed a relationship between progesterone and baseline sIgA, but not with the PostVideo1 sample or the delta between the two samples.

We investigated proactive immune responses of the oral mucosae in relation to visual cues of respiratory diseases. Our null results regarding differences across the menstrual cycle should however not be generalized for the whole body, as oral immunoglobulin levels may not correlate with cervical immunoglobulin levels [30]. A future study on cervical proactive immune responses with other stimuli and questionnaires that relate to sexual disgust – one aspect of disgust that seems to be increased in the luteal phase [12] – might be important to get a greater picture. Also, given the limited sample size of the present study, our null results should not be over-interpreted until supported by replication studies. Lastly, we found that the perceived vulnerability to disease, more specifically germ aversion, was higher in women taking hormonal contraceptives than in those with a natural menstrual cycle, by a large effect. Previous research did not investigate the influence of hormonal contraceptives on this or related questionnaires. Hormonal contraceptives may increase the susceptibility to cervical infections, supposedly due to a down regulation in certain immune markers [13,25,40]. However, whether hormonal contraceptives downregulate immune responses to respiratory diseases, which then might be compensated by enhanced germ aversion and health anxiety currently remains elusive. In our study we found no evidence for a downregulated sIgA baseline in the women that used hormonal contraceptives compared to women who did not. It has previously been speculated that hormonal contraceptives make women more vulnerable to anxiety disorders [34]. This might also explain enhanced anxiety about germs and potential pathogens, without necessitating an actual decline in immunocompetence.

5. Limitations

It can be argued that effects of the menstrual cycle should ideally be tested in a within-subject design. However, in our case the anticipation effect for the second test session might have had a significant influence on sIgA secretion prior to the actual stimulation with a disease video. Wallen and Rupp [57] found in their study on the influence of menstrual cycle phase on interest in sexual stimuli that, if women started their test protocol in the high estradiol state of the late follicular phase, they showed not only increased interest in the sexually explicit photos there, but this effect was also transferred to the other cycle phases. However, testing a within-subject effect in a between-subjects design may require a bigger sample size [17]. While our study had a sample size determined a priori by a power analysis, it may still be important to test a bigger sample to ensure that the targeted differences weren't too small to be detected in our sample. Nevertheless, our results provide preliminary evidence that the menstrual cycle may not be a significant driving factor for intraindividual differences in sIgA release and disgust. This is also in line with other studies that assessed larger samples [27,38,50]. Therefore, we believe that cycle phase and intake of hormonal contraception can be disregarded as potential confounds in future studies on the proactive immune response. Furthermore, Diekhof et al. [7] found that between-subjects design studies can replicate previous results from within-subject designs, even with relatively small sample sizes.

The data for this study was collected during the COVID-19 pandemic. Therefore, a heightened awareness and perceived vulnerability [51] regarding disease-related stimuli might have decreased possible effects throughout the cycle, particular in case of the small hormonal effects that are commonly found in menstrual cycle research. Further, data had to be collected remotely, which might have affected attention while viewing the disease video, thus resulting in lower effects. However, attention to video content was tested through several validation questions and the significant increase in sIgA across groups showed that participants must have paid attention.

Another aspect that represents a limitation of the present study, is that hormonal contraceptive medications and application methods differed between individuals, as shown in Supplementary Table 1. Unfortunately, this could not be avoided, because it was difficult to recruit women at all during the pandemic, even for remote testing. Further, financial resources and temporal availability of student assistants were limited as well. Therefore, the HC sample was very heterogeneous with some women using intrauterine devices that contained only levonorgestrel, but no ethinyl estradiol, while others received high ethinyl estradiol together with other progestins. Future studies therefore have to more carefully recruit women taking the same kind of hormonal contraceptives containing comparable amounts of ethinyl estradiol and progestins.

Lastly, we would like to acknowledge that salivary immunoassays have lately been criticized for possible lack of validity to estimate cycle

phases [1]. In case of the salivary steroid hormones progesterone and estradiol, immunoassays are still common practice, and given the lack of better, reasonable alternatives we used this method here. This was because blood could not be drawn remotely. Additionally, tandem mass spectrometry, such as LC-MS/MS, which is the gold standard for analyses of blood and salivary cortisol, has not yet been validated for estradiol in saliva and also the analysis of progesterone or other sex steroids in saliva through LC-MS/MS can only be achieved by a handful of labs around the world. It is thus not standard methodology when it comes to saliva samples, and thus immunoassays still remain the method-of-choice. In addition, our hormone samples were only used in combination with the backward-counting method for determination of cycle phase, which probably led to a higher accuracy than using either counting methods or hormone levels alone, as most previous studies did.

6. Conclusion

In the present study we found no significant effect of cycle phase on the proactive immune response in saliva, or on associated proactive behavioral mechanisms such as disgust. While the observed u-shaped association between progesterone and baseline sIgA needs further investigation (and especially the dip of the function around ovulation), our null results on the group level are in line with previous findings [27,38,41,50]. Yet, studies with bigger samples are certainly needed to replicate these null findings, as we cannot rule out that our relatively small between-subjects sample was slightly underpowered.

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Conflict of interest

The authors report no conflict of interest.

Data availability

The data that support the findings of this study are openly available under https://osf.io/yzu95/.

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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.repbio.2024.100880.

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2.3.1 Supplementary material and methods of Chapter III

Supplementary: Influence of female sex hormones on proactive behavioral and physiological immune parameters

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Supplementary Instructions for saliva sampling 1

Original instructions in German

Am Morgen des Testtages:

Es werden insgesamt 7 Proben gesammelt. Die ersten drei Proben sollen morgens nach dem Aufstehen über den Zeitraum einer Stunde genommen werden. D.h. Probe 1 direkt nach dem Aufstehen, Probe 2 ca. 30 Minuten danach und Probe 3 ca. 1 Stunde nach dem Aufstehen. Während dieser Zeit sollte nichts gegessen werden und auch keine Getränke oder Zigaretten konsumiert werden. Wasser darf nach der 1. Probe und bis zu 5 Minuten vor der 2. getrunken werden. Sie dürfen sich auch direkt nach Abgabe der ersten Probe am Morgen die Zähne putzen.

Am Nachmittag des Testtages:

Die weiteren 4 Proben werden während des Online-Experiments zu genau festgelegten Zeitpunkten abgegeben. Bitte verzichten Sie ab 1 Std. vor Beginn des Online-Experimentes auf Nahrung und koffeinhaltige Getränke (z.B. Tee, Kaffee). Kurz gesagt, bleiben Sie „nüchtern". Sie dürfen lediglich bis 10 Minuten vor der ersten Probenentnahme Wasser trinken. Zudem sollte der Mund 10 Minuten vor der ersten Probenentnahme einmal mit Wasser gespült werden.

English translation

On the morning of the test day:

A total of 7 samples are collected. The first three samples are to be taken in the morning directly from wake-up time, and over the period of one hour. This means sample 1 will be taken directly after waking up, sample 2 approximately 30 minutes afterwards and sample 3 approximately 1 hour after waking up. During this time, nothing should be eaten and no drinks or cigarettes should be consumed. Water may be drunk after the 1st sample and up to 5 minutes before the 2nd. You may also brush your teeth immediately after providing the first sample in the morning.

In the afternoon of the test day:

The other 4 samples will be given at precisely specified times during the online experiment. Please refrain from food and caffeinated beverages (e.g. tea, coffee) from 1 hour before the start

of the online experiment. You may only drink water until 10 minutes before the first sample collection. In addition, the mouth should be rinsed once with water 10 minutes before the first sample is taken.

Supplementary Table 1: Detailed information on hormonal contraceptives (HC) taken by the Hormonal Contraception group in the study.

		Estrogen		*Progestin*	
ID	Kind of HC	Kind	Daily Concentration in mg	Kind	Daily Concentration in mg
600	Pill	Estradiol	1.500	Nomegestrol acetate	2.500
601	Pill	Ethinyl estradiol	.030	Dienogest	2.000
602	Pill	Ethinyl estradiol	.020	Levonogestrel	.100
603	Pill	Ethinyl estradiol	.030	Levonogestrel	.150
604	Pill	Ethinyl estradiol	.030	Dienogest	2.000
605	Pill	Ethinyl estradiol	.020	Levonogestrel	.100
614	Pill	Ethinyl estradiol	.015	Etonogestrel	.120
616	Pill	Ethinyl estradiol	.035	Cyproteron acetate	2.000
619	Pill	Ethinyl estradiol	.020	Levonogestrel	.100
620	Pill	Ethinyl estradiol	.300	Dienogest	2.000
621	Pill	Ethinyl estradiol	.020	Levonogestrel	.100
625	Pill	Ethinyl estradiol	.030	Chlormadinon acetate	2.000
627	Pill	Ethinyl estradiol	.020	Levonogestrel	.100
628	Pill	Ethinyl estradiol	.030	Chlormadinon acetate	2.000
609	Ring	Ethinyl estradiol	.015	Etonogestrel	.120
612	Ring	Ethinyl estradiol	.015	Etonogestrel	.120
626	Ring	Ethinyl estradiol	.015	Etonogestrel	.120
629	Ring	Ethinyl estradiol	.015	Etonogestrel	.120
610	IUD			Levonogestrel	.009
615	IUD			Levonogestrel	.006

IUD: Intrauterine Device

Supplementary Table 2: Results of the Kolmogorov-Smirnov Test over the relevant variables

Variable	*D*	*N*	*p-value*
Estradiol	0.157	81	<.001
Progesterone	0.279	81	<.001
Baseline	0.128	81	.003
Post-Video 1	0.184	81	<.001
Post-Video 2	0.227	81	<.001
Post-Video 3	0.204	81	<.001
Sum Score Vulnerability to Disease	0.079	81	.200*
Subscale Germ Aversion	0.076	81	.200*
Subscale Perceived Infectability	0.088	81	.200*
Sum Score Disgust	0.082	81	.200*
Subscale Contamination Disgust	0.128	81	.003
Subscale Core Disgust	0.106	81	.031
Contagion Rating	0.084	81	.200*
Illness Rating	0.132	81	.002
Disgust Rating	0.198	81	<.001

*This is a lower bound of the true significance

Supplementary Table 3: Linear (a) and quadratic (b) regression analysis of the relationship between rank transformed estradiol and sIgA samples

A) Linear

Sample	R^2	*F*	*df1*	*df2*	*p-value*
Baseline	.049	2.276	1	44	.139
Post-Video 1	.026	1.183	1	44	.283
$\Delta sIgA_1$*	.002	.069	1	44	.794

B) Quadratic

Sample	R^2	*F*	*df1*	*df2*	*p-value*
Baseline	.057	1.306	2	43	.281
Post-video 1	.027	.590	2	43	.559
$\Delta siga_1$*	.005	.113	2	43	.893

* $\Delta sIgA_1$ = Post-Video 1 – Baseline

2.4 Chapter IV: Visual Cues of Respiratory Contagion: Their Impact on Neuroimmune Activation and Mucosal Immune Responses in Humans

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Visual cues of respiratory contagion: Their impact on neuroimmune activation and mucosal immune responses in humans

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ABSTRACT

This study investigated the neural correlates of perceiving visual contagion cues characteristic of respiratory infections through functional magnetic resonance imaging (fMRI). Sixty-two participants (32f/ 30 m; ~25 years on average) watched short videos depicting either contagious or non-contagious everyday situations, while their brain activation was continuously measured. We further measured the release of secretory immunoglobulin A (sIgA) in saliva to examine the first-line defensive response of the mucosal immune system. Perceiving sneezing and sick individuals compared to non-contagious individuals triggered increased activation in the anterior insula and other regions of the neuroimmune axis, that have been implicated in the somatosensory representation of the respiratory tract, and further led to increased release of sIgA. In line with predictions, this contagion cue-related activation of the insula was positively correlated with both perceived contagiousness and disgust evoked by the videos, as well as with the mucosal sIgA response. In contrast, the amygdala exhibited heightened activation to all videos featuring humans, regardless of explicit signs of contagion, indicating a nonspecific alertness to human presence. Nevertheless, amygdala activation was also correlated with the disgust ratings of each video. Collectively, these findings outline a neuroimmune mechanism for the processing of respiratory contagion cues. While the insula coordinates central and peripheral immune activation to match the perceived contagion threat, supposedly by triggering both increased sIgA release and contagion-related cognitions, the amygdala may rather act as an alerting system for social situations with a heightened transmission risk. This proactive neuroimmune response may help humans to manage contagion risks, that are difficult to avoid, by activating physiological and cognitive countermeasures in reaction to typical symptoms of respiratory infection, which prepares the organism for subsequent pathogen exposure.

1. Introduction

Throughout human history, communicable diseases, such as respiratory viral infections, constituted a significant cause of mortality (Shaw-Taylor, 2020). The constant threat of pathogen transmission thereby led to the evolution of several potent innate and adaptive immunological mechanisms in response to infection, which form the physiological immune system (PIS). While the PIS is highly effective at handling pathogen infestation, its recruitment is metabolically costly and could temporally incapacitate, permanently damage or even kill the organism (Pacheco-López and Bermúdez-Rattoni, 2011). To reduce the probability of pathogen contact in the first place, humans own an additional repertoire of proactive cognitive, affective and behavioral adaptations, the so called behavioral immune system (BIS). The BIS comprises mechanisms such as avoidance behavior, contamination disgust or perceived disease vulnerability, which are triggered by situations of increased contagion probability (Curtis et al., 2004; Kavaliers et al., 2019; Mortensen et al., 2010; Schaller, 2011). Similar to the PIS, activation of the BIS also comes with specific opportunity costs. Heightened avoidance behavior and feelings of disgust, while helpful in preventing infection, limit chances to acquire resources, status, or potential mates and may inadvertently lead to social isolation (Gangestad et al., 2016; Oaten et al., 2009; Schaller, 2011). It has thus been proposed that the coordinated interaction of the BIS and PIS is obligatory to optimally balance the cost/benefit ratio between them (Cepon-Robins et al., 2021; Gassen et al., 2018; Keller et al., 2022; Miller and Maner,

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2011; Schaller and Duncan, 2007; Stevenson et al., 2015).

The coordinated interaction between the proactive BIS and the reactive PIS becomes particularly important in the highly contagious contexts that are typically associated with respiratory viral infections such as influenza or the coronavirus disease (COVID-19). Respiratory infections primarily spread through airborne aerosols and particles emitted by sneezing, coughing, talking or even breathing, which easily enter the respiratory tract through the oral and nasal cavities (Zhou et al., 2021). Airborne pathogen transmission is almost impossible to avoid, and protection against it thus at best demands the preparatory activation of both cognitive-behavioral and immunological responses. And indeed, there is initial evidence suggesting that merely perceiving visual cues of an imminent contagion threat (e.g., viewing a sneezing person) may prompt an active boost in the mucosal immune response. This involved the increased release of secretory immunoglobulin A (sIgA), Tumor Necrosis Factor α (TNF-α) and albumin in saliva in response to respiratory contagion cues, such as videos displaying sneezing and coughing persons, or was triggered by pictures of other disease-indicators, like skin rashes or typical hospital settings (Brown et al., 2014; Keller et al., 2024, 2023, 2022; Keller and Diekhof, 2024; Stevenson et al., 2015, Stevenson et al., 2012). Most notably, the immune response thereby occurred proactively, even without subsequent pathogen contact of the mucosae. In addition, an increased cytokine reaction to immunological challenge in blood has also been observed following the mere visual stimulation with disease indicators (Schaller et al., 2010).

The ability to adapt immune responses to sensory cues in the environment enables immune cells to tune their responses to a large variety of contexts and conditions (Natoli and Ostuni, 2019). Through the coordinated interaction between the central nervous system and the PIS sensory information thereby reaches central brain regions that have an integrative function in neuro-immune interactions, which can modulate cognitive, behavioral and other bodily responses to cope with immunological challenges (Blalock, 2005; Chesné et al., 2019; Dantzer, 2018; Dantzer et al., 2000; Goehler et al., 2000; Hu et al., 2022; Kerezoudis et al., 2022; Pacheco-López and Bermúdez-Rattoni, 2011). Previous functional magnetic imaging (fMRI) studies consistently suggested that both the insula and the amygdala are implicated in the manifestation of systemic inflammatory responses such as fatigue, anxiety and lowered mood as central aspects of "sickness behavior" (Harrison et al., 2009b, 2009a; Lekander et al., 2016; Månsson et al., 2022). Rodent studies support these findings (Doenlen et al., 2011) and further suggest that the insula may be particularly important for the acquisition of immune conditioning (Pacheco-López et al., 2005; Ramírez-Amaya et al., 1996; Ramírez-Amaya and Bermúdez-Rattoni, 1999) and immunological memory (Koren et al., 2021). Apart from that, in humans both the insula and the amygdala have been found to respond to disgusting or otherwise repulsive stimuli (Hayes et al., 2014; Kipps et al., 2007; Schäfer et al., 2005; Wicker et al., 2003). Notably, the insula also responded to the observation of disgust responses in others, which suggests an additional role in the detection of specific malaise cues (Jabbi et al., 2008; Wicker et al., 2003). Regenbogen et al. (2017) found increased activation in the posterior insula when participants perceived photographs of faces (and/or the smell of the respective person) exhibiting the subtle sickness signs of an inflammatory endotoxin challenge [but see Leschak et al., 2022, who failed to find insular activation in response to a similar set of "sick" faces]. Given this evidence, it seems plausible to assume that the insula – as a sensory-immunological integration hub (Schiller et al., 2021) – may also be responsive to the specific class of respiratory contagion cues. This is, because contagion indicators, such as sneezing or coughing, are highly predictive of subsequent pathogen exposure and indicate the imminent need for enhanced immune activation. To what extent the human insula might also be implicated in modulating preemptive mucosal immune responses, such as those triggered by the visual cues of respiratory contagion (e.g., Keller et al., 2024, 2023, 2022; Keller and Diekhof, 2024), is still unclear. A functional link is very likely, given the insula's role in immune retrieval (Koren et al., 2021) and immune conditioning (Schedlowski and Pacheco-López, 2010), further suggesting that insular responses to discriminatory contagion cues may also trigger the associated immunological cascade.

The aim of this preregistered neuroimaging study was to fill this gap of knowledge by particularly focusing on neural processing of typical visual respiratory contagion cues that are often encountered in real life. Contagion cues, such as sneezing or coughing, imply that subsequent pathogen exposure is inherently difficult to evade, thereby necessitating a proactive mucosal immune response as a quasi-obligatory measure. Here, we used fMRI to assess brain activation during the perception and evaluation of contagion videos depicting real life contagious contexts that included a mix of sneezing persons and persons with other typical flu-like symptoms such as looking feverish, shivering, and being in pain (*Contagion* condition). Visually matched videos of healthy humans (*Healthy Humans* condition) and videos with similar background settings, but without humans (*Low-Level* videos) were used as the two control conditions. During the fMRI scan, the videos were not only passively viewed, but were also subsequently evaluated with regard to their contagion and disgust-eliciting potential. Similar to the design of Wabnegger et al., (2021) we presented 16 videos in each of the three conditions. A video was shown twice in a pseudorandomized counterbalanced sequence and with temporally jittered onsets (i.e., video conditions and the two rating categories of contagion and disgust-eliciting potential were equally distributed across two functional runs). This design was intended to separate the actual video perception from its evaluation, while also putting a focus on the contagion-related aspects of the videos. By this, we wanted to ensure a more realistic perception of the contagion videos in the artificial scanner environment and thus successfully stimulate a proactive mucosal immune response, as shown previously (Keller et al., 2022, 2023). We collected three saliva samples to investigate the sIgA secretion in saliva before, during and after stimulation with contagion-related content. We expected increased activation of the insula and amygdala, and of other functionally connected regions of the neuroimmune axis, when participants watched the *Contagion* videos compared to the *Healthy Human* and *Low-Level* videos. Additionally, we predicted a relationship between contagion-related activation of regions from the neuroimmune axis and the subjective ratings of their contagion- and disgust-eliciting potential as well as with the increase of sIgA from baseline to post-stimulation (see: https://osf.io/usbxw/).

2. Materials & Methods

2.1. Participants

Participants were recruited on university campus, through online advertisements and social media. Participants had to be right-handed and currently healthy. Further, exclusion criteria consisted of a history of psychiatric or neurological disorders, a chronic disorder of the immune or the hormone system (e.g., autoimmune disorders, diabetes), allergies (e.g., asthma, allergic rhinitis), or cancer. Further, persons were not allowed to participate if they were pregnant and or used medications around the time of testing (except for hormonal contraceptives), or had been using antibiotics in the two weeks prior to the test. Lastly an operation in the last 2 months, metal implants or non-removable metal parts on/in the body that would pose a health risk in the MR-scanner were also exclusion criteria.

Participants had to be between 18 and 35 years old like in comparable psychoimmunological studies. As we stated in the preregistration of this study (https://osf.io/usbxw/) we aimed to test 60 healthy participants, which was similar to the sample size of comparable fMRI studies investigating the perception of emotional stimuli (e.g., Wabnegger et al., 2021). We reached this goal by testing a total of 67 participants. Of these, five had to be excluded from all analyses for various reasons: (1.) technical difficulties with the stimulation computer during

the fMRI-Scan (n = 1), (2.) excessive head movement by more than 2.5 mm in one direction during the functional scans (n = 3), and (3.) an incidental medical finding in the anatomical scan (n = 1). This resulted in a final sample of 62 participants. The study was approved by the local ethics committee *"Ethikkommission der Ärztekammer Hamburg"* (2022–100903-BO-ff) and conformed with the Declaration of Helsinki. Participants provided written informed consent and were paid for participation.

2.2. Procedure

Prescreening. Before the actual test day, participants completed an online questionnaire in which they provided demographic data on aspects such as age and gender as well as their health history in order to estimate study eligibility. Further, participants gave detailed information relevant for the fMRI-scan, e.g., metal implants, right handedness and correction of eyesight. After that, participants filled in the modified version of the Revised Disgust Scale (Olatunji et al., 2007) to measure trait disgust sensitivity. We thereby used the 17 items comprising the two subscales of core disgust and contamination disgust. Of these, eight items encompassed statements like "*I might be willing to try eating monkey meat, under some circumstances.*", for which participants indicated their agreement on a 5-point Likert-scale (from 0 = '*Strongly disagree*' to 4 = '*Strongly agree*'). The rest of the items, such as: "*While you are walking through a tunnel under a railroad track, you smell urine.*", required an explicit disgust rating from 0 = '*Not disgusting at all*' to 4 = '*Extremely disgusting*'. Finally, participants rated their perceived Vulnerability to Disease (VTD) (Duncan et al., 2009). The 15 items of the VTD included statements like "*In general, I am very susceptible to colds, flu and other infectious diseases.*" and "*I prefer to wash my hands pretty soon after shaking someone's hand.*", associated with the two subscales of germ aversion and perceived infectability, which the participants had to evaluate with a 7-point Likert-scale (1 ='*Strongly disagree*' to 7 = '*Strongly agree*').

Test day. The participants were all tested in the afternoon (between 12p.m. and 17p.m.) to account for the circadian rhythm of mucosal immune responses (Plangsangmas et al., 2020). The data collection took place between February 2023 and August 2023. After arrival at the MR-facility (*University Hospital Hamburg Eppendorf, Germany*), the participants attended a short medical interview with an MR-physician, who approved study eligibility.

The MR-protocol started with the anatomical and the field map scans, while the participants watched a nature video during which they were instructed to relax (duration= ~8min). Directly after this initial relaxation phase, the participants provided the first saliva sample for later sIgA analysis (*Baseline* sample; duration = 4 min). The protocol proceeded with the first functional run (Run 1), during which the participants watched a total of 48 videos of the following three conditions: (1.) *Contagion* videos (see Fig. 1a) with sneezing people or people showing other flu-like symptoms, (2.) videos showing *Healthy Humans* (see Fig. 1b) in comparable situations as (1.), and (3.) *Low-Level* control videos (see Fig. 1c) showing background settings matching those from the videos of (1.) and (2.). The video sequences were pseudorandomized

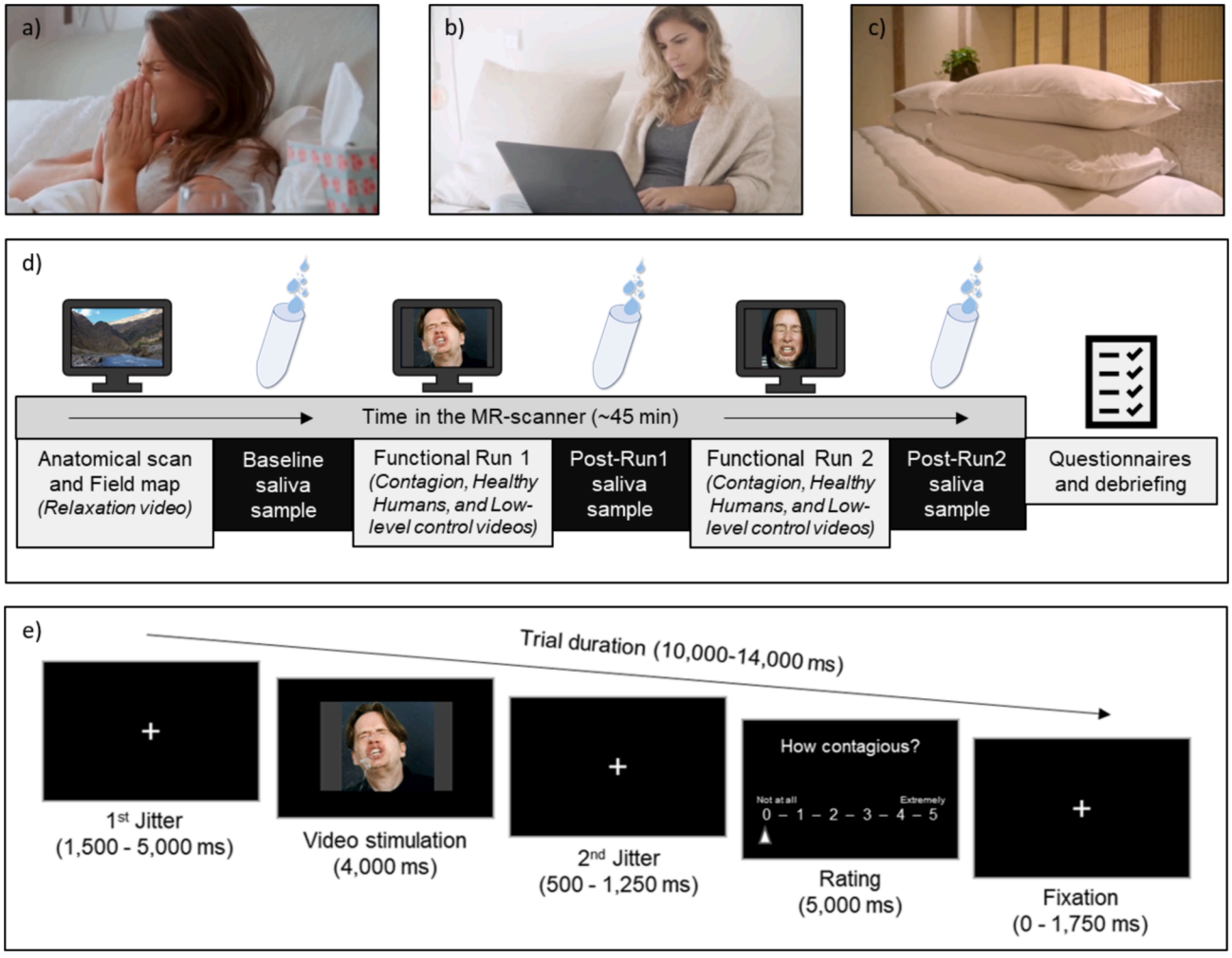


Fig. 1. Experimental procedure. (a) Screenshot from exemplary contagion video. (b) Screenshot of a matched situation showing a healthy human. (c) Screenshot from the corresponding low-level control video. (d) Schedule of experimental interventions, and (e) exemplary trial with timings and delay variations.

and counter-balanced for transitions between video conditions (see SI Appendix, Supplementary Table S4 for trial sequence). In Run 1, eight different videos were shown twice per condition. Following the presentation of a video and after a variable blank-screen delay, the video was directly rated for its contagion (*'How likely do you think it is to become infected in the situation shown?'*) or disgust potential (*'How disgusted are you by the situation shown?'*). Questions were answered on a 6-point Likert-scale (0 = *'Not at all'* – 5 =*'Extremely'*). For this, the participants utilized two buttons of the *4 Button Bimanual* button box from *Current Design* (www.curdes.com) to move a slider up or down the scale. The starting position of the slider (left or right of the scale) was counterbalanced between runs and participants (i.e., if the start position was on the left during the first run, it was on the right during the second, or vice versa). Since each video was shown twice within a functional run, the participants either received the disgust- or contagion-related question first, while the other one was shown after the second display of the same video. The sequence of the two questions was also pseudorandomized and counterbalanced across video conditions (see Appendix, Supplementary Table S4). Therefore, the participants could not exactly predict, whether they would have to rate the video according to its disgust or contagion potential (also see Fig. 1d & e for details of the experimental procedure and an exemplary trial). After the first run, the participants provided the second saliva sample (*Post-Stimulation 1*; 4 min), and proceeded with functional Run 2, during which another eight videos per condition, each shown twice in a pseudorandomized and counter-balanced way, were rated for their disgust and contagion potential. After Run 2, the last saliva sample (*Post-Stimulation 2*; 4 min) was collected and the participants left the scanner. They were then transferred to a quiet room, where they answered post-scanning questionnaires: Firstly, they were are asked to recall the experience during the anatomical scan and answered questions of the Simulator Sickness Questionnaire (SSQ) (Kennedy et al., 1993) in regards to their feelings in the MR-scanner. Utilizing a 4-Point Likert-scale (from 1 = '*Not at all*' to 4 = '*Strongly*') they described if they felt certain symptoms such as fatigue, nausea or headache (16 items). Afterwards, they were asked to reflect on the *Contagion* videos of the two functional runs utilizing an '*Absolute Recall Task*' to examine the emotional state, related to watching the videos using 6 statements, such as "*How strong was your feeling of disgust, while watching the* video?"'. The statements about feelings had to be rated on a 9-point Likert-scale from 0 = '*Not at all*' to 8 = '*Completely*'. This absolute recall of feelings depicted an identical number of negative (e.g., stress, disgust) and positive feelings (e.g., amusement, inspiration). Additionally, we focused on the physical urges and feelings during the videos. For this we modified the pathogen defense items from the inventory by Kupfer et al. (2021). We separated the items into two composite scores: The *Gastric composite score* included the items '*I felt sick during the video*', '*I felt like I could vomit*', '*I felt a physical sensation in my stomach*'. The *Respiratory composite score* included the items '*I felt a physical sensation in my throat*', '*I had a lot of saliva in my mouth during the video*', '*I felt the urge to cover my mouth and nose during the video*', '*I felt dirty during the video*', '*I had the urge to wash myself*', '*I had the feeling I could be infected during the video*', '*I had light body aches during the video*', and '*I felt weak and ill during the video*'. All items were rated on an 8-point scale ranging from 0 = '*Not at all*' to 7 = '*Very strongly*'.

2.3. Saliva samples

During the test session, three saliva samples were collected head supine with Salivette swabs (Sarstedt). The swab was placed inside the mouth of the participant, i.e., between the dental arch and the cheek, where it was left for 4 min to collect secreted saliva. The participant was instructed not to chew on the swab, which was very important, since chewing may induce increased sIgA release (Proctor and Carpenter, 2001). After collection, swabs were put on dry ice, were transferred to our inhouse lab and weighed for the later calculation of the secretion rate of sIgA according to the formula: $Secretion\left(\frac{\mu g}{min}\right) = \frac{Volume(mL)}{Time(min)} * Concentration\left(\frac{\mu g}{mL}\right)$. Afterwards the samples were frozen at −20 °C. For further analysis, all samples were anonymized and sent to an external laboratory (*MVZ Volkmann Laboratory*, Karlsruhe, Germany), where they were analyzed with an immuno-nephelometric analysis (Atellica® NEPH 630 System) to determine the concentration of sIgA in saliva.

2.4. fMRI parameters

Neuroimaging was conducted on a 3 T Magnetom-Prisma MR-scanner (Siemens Healthcare) at the *University Hospital Hamburg Eppendorf, Germany*. The scanner protocol started with an anatomical scan, i.e. a typical high-resolution T1-weighted image, that was acquired with the 3D magnetization prepared gradient echo sequence (MPRAGE) (isotropic voxel with sub-millimetric size, water selective excitation and acceleration factor of 2 using GRAPPA) and a field map scan. Functional images were acquired with a T2-weighted, gradient-echo, echoplanar imaging (EPI) sequence with a BOLD contrast using a 64-channel head coil, with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle = 60°, field of view = 224 mm, number of slices = 62 slices, parallel to the anterior-posterior commissure obtained in an interleaved acquisition order, slice-thickness = 2 mm, no gap.

2.5. Data analysis

Following our preregistered analysis protocol (https://osf.io/usbxw/), we utilized Matlab and Statistical Parametric Mapping (SPM12, https://www.fil.ion.ucl.ac.uk/spm) for data preprocessing and analysis of neuroimaging data. Preprocessing included co-registration, realignment and unwarping, correction for slice-time acquisition differences and low-frequency fluctuations, normalization into standard stereotactic space [skull-stripped EPI template of the Montreal Neurological Institute (MNI)], and spatial smoothing with an isotropic Gaussian kernel filter of 6 mm full-width half-maximum. In the first-level single-subject analysis we specified an event-related model with the regressors: *Contagion* (onsets of the *Contagion* videos), *Healthy Human* (onsets of the *Healthy Human* videos), *Low-Level* (onsets of the *Low-level* videos), *Contagion Rating* (onsets of contagion questions parametrically modulated by the individual rating responses to each question), *Disgust Rating* (onsets of disgust questions with responses to each question as parametric modulation), as well as *Dummy* (onset of the two dummy videos at the beginning of each functional run) and *End* (onset of the final screen signaling the end of a run) as the two regressors-of-no-interest. Based on these regressors, we defined linear t-contrasts to assess brain activation elicited by the perception of the *Contagion* videos compared to their matched control conditions (*Healthy Human* or *Low-Level* videos), while the two parametrical regressors (*Contagion Rating* and *Disgust Rating*) were each contrasted against the implicit baseline. These contrasts were then used in the second-level *Random-Effects analysis* to assess brain activation across group. A whole-brain correction for multiple testing using the family-wise error (FWE) at peak level, thresholded at $p < 0.05$, no minimum clustersize, was applied to all analyses.

Following the pre-registered whole-brain analyses, we performed an exploratory region of interest analysis that was not included in detail in the preregistration. For this, we defined four anatomical regions of interest (ROI) based on the AAL3 toolbox (Rolls et al., 2020). These comprised the complete anatomical volumes of the two key regions of the neuroimmune axis, the left and right insula and amygdala, respectively. We used the complete anatomical volumes as ROIs in order to reduce the bias that occurs when restricting a ROI to a specific activation maximum from the second-level whole-brain analysis (e.g., the small cluster in the anterior insula). From these anatomical ROIs we extracted the parameter estimates of the key contrast of *'Contagion > Healthy*

Human' with the MarsBar Toolbox (Brett et al., 2002). The extracted parameter estimates were then correlated with the sIgA secretion rates from each sampling and the delta between the last and the first sample ($\Delta sIgA_{Overall}$ = *Post-Stimulation 2 – Baseline*), the average contagion rating, the average disgust rating and the interoceptive composite scores (i.e., the Gut- and the Respiratory- composite scores) from each participant. In addition, we also correlated the parameter estimates with trait disgust and perceived disease vulnerability.

Behavioral and physiological data were analyzed with IBM SPSS (Version 29.0) with a statistical threshold of $p < 0.05$ (two-tailed). These data can be found in the Supplementary Excel file "*Data_Table_SPSS*". To determine if data conformed to a Gaussian normal distribution we first utilized a Kolmogorov-Smirnov test for each variable (KS-test). A significant deviation from normal distribution was thereby assumed at $p < 0.05$ (for detailed results of the KS-test see SI Appendix, Supplementary Table S5). Correlations between parameter estimates and the variables outlined above were calculated with either Pearson or Spearman-Rank correlations depending on the result of the respective KS-test. We further used a GLM with repeated measures for the average rating of each *Condition* (*Contagion, Healthy Human, Low Level*) as within-subject factor. Since the rating data were non-parametric, we employed Wilcoxon-Signed-Rank tests as post-hoc tests. For the investigation of the change of sIgA secretion across the experiment we also used a GLM with repeated measures with *Sample* (*Baseline, Post-Stimulation 1, Post-Stimulation 2*) as within-subject factor. Again, we used Wilcoxon-Signed-Rank tests as post-hoc test. Graphs were created with RStudio (Version 2023.09.0).

3. Results

Altogether, data of 62 participants (32f/ 30 m; mean age = 24.85 years, SD = 4.53 years) were included in the main analyses of behavioral and neuroimaging data. For the sIgA-related analyses we had to exclude the data of 15 participants, who lacked sufficient material for the nephelometric analysis in at least one of their saliva samples (missing values). All analyses that included sIgA thus relied on a smaller sample of 47 persons.

3.1. Video rating

We found a significant influence of Video condition (*Contagion, Healthy Human, Low-Level*) on the contagion rating ($F_{(2,122)} = 601.50$, $p < 0.001$, $p\eta^2 = 0.908$). In general, the *Contagion* videos were rated as significantly more contagious than the *Healthy Human* ($z = -6.85$, $p <$

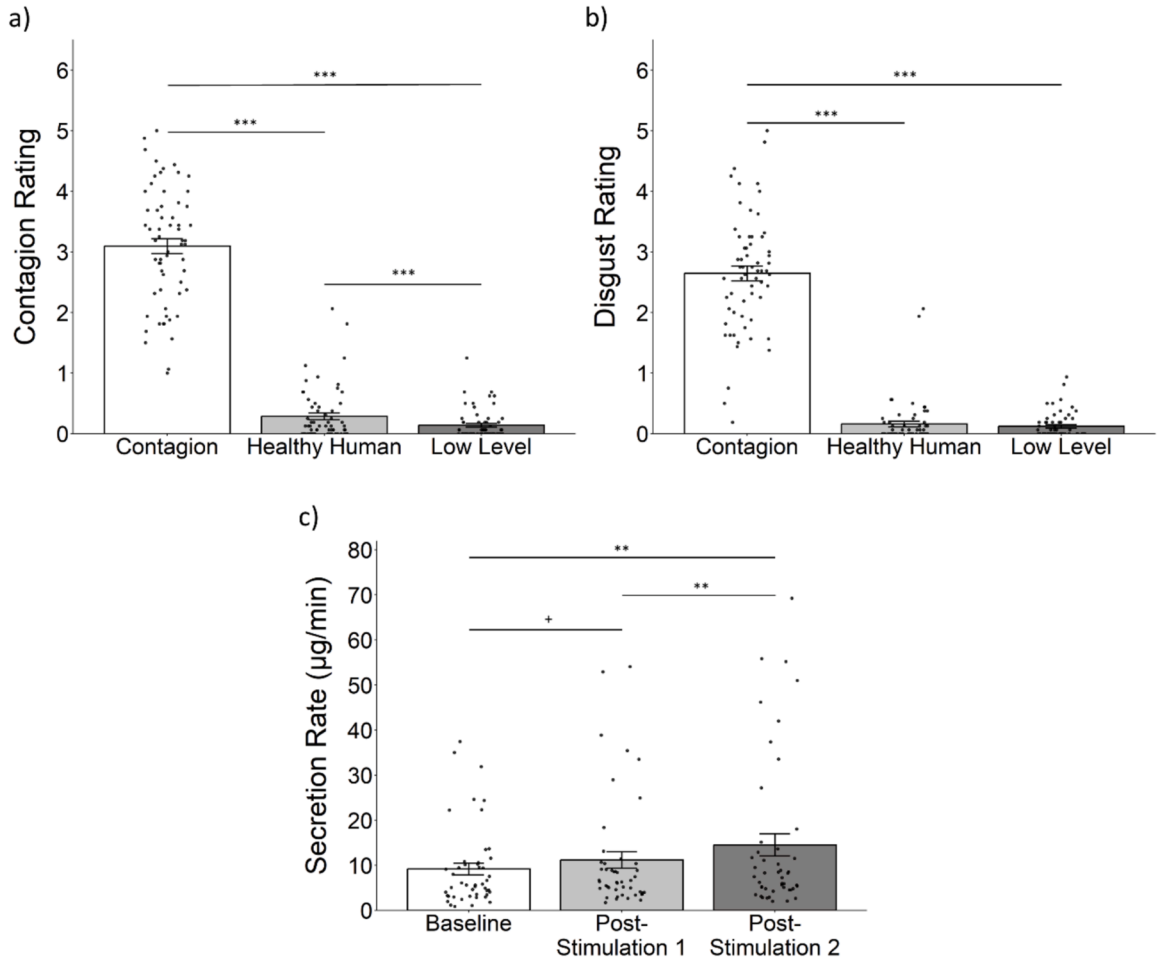


Fig. 2. Average ratings of the three video conditions and sIgA secretion rate across the experiment. (a) Contagion rating of the three video conditions ($p < 0.05$; two-tailed, N = 62). (b) Disgust rating ($p < 0.05$; two-tailed, N = 62). (c) sIgA change from baseline sample, taken after the relaxation period, to the first sample taken after functional run 1 (Post-Stimulation 1) and the second sample taken after completion of run 2 (Post-Stimulation 2) ($p < 0.05$; two-tailed, N = 47). Figure depicts mean values and standard errors as well as real data points. Statistical significance is indicated by asterisks (***<.001; **<.010), while a statistical trend ($p < 0.10$) is indicated by a cross.

0.001, $\eta^2 = 0.76$) and the *Low-Level* videos (z = -6.85, p < 0.001, $\eta^2 = 0.76$). Interestingly, the *Healthy Human* videos were also rated as significantly more contagious than the *Low-Level* videos (z = -3.95, p < 0.001, $\eta^2 = 0.78$) (see Fig. 2a).

In the second GLM of the disgust-eliciting potential of the videos, we also found a significant influence of video condition on the disgust rating ($F_{(2,122)} = 473.21$, p < 0.001, $p\eta^2 = 0.886$). The *Contagion* videos were rated as more disgusting than the *Healthy Human* (z = -6.85, p < 0.001, $\eta^2 = 0.76$) and the *Low-Level* videos (z = -6.85, p < 0.001, $\eta^2 = 0.76$). In contrast to the contagion rating, we found no significant difference between the *Healthy Human* and *Low-Level* videos (z = -0.53, p = 0.597, $\eta^2 = 0.01$) (Fig. 2b).

3.2. Changes in sIgA secretion during fMRI

The GLM found a significant influence of *Sample* (*Baseline, Post-Stimulation 1, Post-Stimulation 2*) on the sIgA secretion rate ($F_{(2,92)} = 5.19$, p = 0.015, $p\eta^2 = 0.101$). Accordingly, sIgA significantly increased from *Baseline* to *Post-Stimulation 2* (z = -2.66, p = 0.004, $\eta^2 = 0.15$), and also rose from *Post-Stimulation 1* to *Post-Stimulation 2* (z = -2.48, p = 0.007, $\eta^2 = 0.13$). However, the increase between *Baseline* and *Post-Stimulation 1* only reached statistical trend level (z = -1.39, p = 0.083, $\eta^2 = 0.04$) (Fig. 2c).

3.3. Neuroimaging

3.3.1. Influence of the perception of the contagion videos on whole-brain activation

In the whole-brain analysis, we wanted to assess changes in brain activation specifically evoked by videos displaying potentially contagious everyday situations (i.e., videos of sneezing humans or of humans with other symptoms indicative of a respiratory disease). For this, we first examined the contrast *Contagion* > *Healthy Human* of the perception phase, which was intended to reveal brain regions specifically responsive to typical symptoms of respiratory infection with a heightened contagion potential. This contrast revealed enhanced bilateral activation in the anterior insula (Fig. 3a), while no significant differences emerged in the amygdala (Table 1).

Apart from the anterior insula, we also identified a big cluster that included the ventral postcentral gyrus and the parietal operculum (i.e., area SII of the somatosensory cortex, Fig. 3b), as well as adjacent parts of the supramarginal gyrus. In addition, we observed increased activation of the nucleus of the solitary tract (NST, Fig. 3c) in the brainstem and of the periaqueductal gray (PAG, Fig. 3d). We also found increased activation of several ventral visual stream areas (i.e., inferior occipital and temporal regions, including the fusiform face area), of frontal regions implicated in cognitive and motor control (i.e., the inferior frontal junction [IFJ] and the pre-supplementary motor area [pre-SMA]), of the cingulate gyrus, the precuneus and the inferior parietal lobe (IPL), as well as the caudate nucleus and the cerebellum. Most of these regions also showed consistent activation in the contrast of *Contagion* > *Low-Level* (see also SI Appendix, Supplementary Table S1 for complete list of activation clusters).

Interestingly, we found increased activation in the amygdala in the contrast of *Contagion* > *Low-Level*, and the amygdala was also significantly activated when comparing the non-contagious *Healthy Human* to the *Low-Level* condition (Fig. 4, Table 1), which did not activate the anterior insula.

Apart from that, the control contrast of *Healthy Human* > *Low-Level* solely showed increased activation in the inferior occipital gyrus and the fusiform face area (For complete list of activation clusters see SI Appendix, Supplementary Table S1).

3.3.2. Association between the contagion rating and whole-brain activation

The whole-brain parametrical analysis of the *Contagion Rating* revealed increased activation in the ventral anterior insula extending into adjacent posterior orbitofrontal cortex when the video content was perceived as more contagious (Table 2). Further, similar to the perception phase of the contagion videos, the subsequent rating of higher contagion potential positively scaled with activation of the PAG, the IFJ, ventral visual stream and face processing areas, and the frontal cortex (see also SI Appendix, Supplementary Table S2 for complete list of activation clusters).

3.3.3. Association between the disgust rating and whole-brain activation

An increased disgust rating of the videos led to increased activation in both the anterior insula and the amygdala (Table 2). Apart from that, we also found increased disgust-related activation of the supramarginal gyrus, the IFJ, ventral visual stream and face processing areas, the frontal cortex and the thalamus (see SI Appendix, Supplementary Table S2).

3.3.4. Correlation between brain activation in key regions of the neuroimmune axis during perception of the contagion videos and increased sIgA secretion during fMRI (ROI-based analysis)

To investigate the relationship of brain activation in the insula and the amygdala with enhanced sIgA secretion during fMRI, we extracted the beta estimates from these key regions of the neuroimmune-axis. The four ROIs thereby comprised the complete volumes of these brain regions in the left and right hemisphere as defined by the anatomical atlas AAL3 (Rolls et al., 2020). Beta estimates were extracted from the key contrast *Contagion* > *Healthy Human,* which best reflects the perception of contagious respiratory symptoms.

When correlating the individual beta values of the four ROIs with the sIgA secretion rate we found positive correlations between the right insula and sIgA secretion for the samples taken *Post-Stimulation 1* (rho = 0.29, p = 0.050) and *Post-Stimulation 2* (rho = 0.39, p = 0.008). Apart from that, we also found a positive correlation between activation of the right insula and the overall sIgA increase during fMRI, i.e., $\Delta sIgA_{Overall}$ (rho = 0.31, p = 0.035), as well as between activation of the left insula and $\Delta sIgA_{Overall}$ (rho = 0.30, p = 0.044, see Fig. 5a, Table 1).

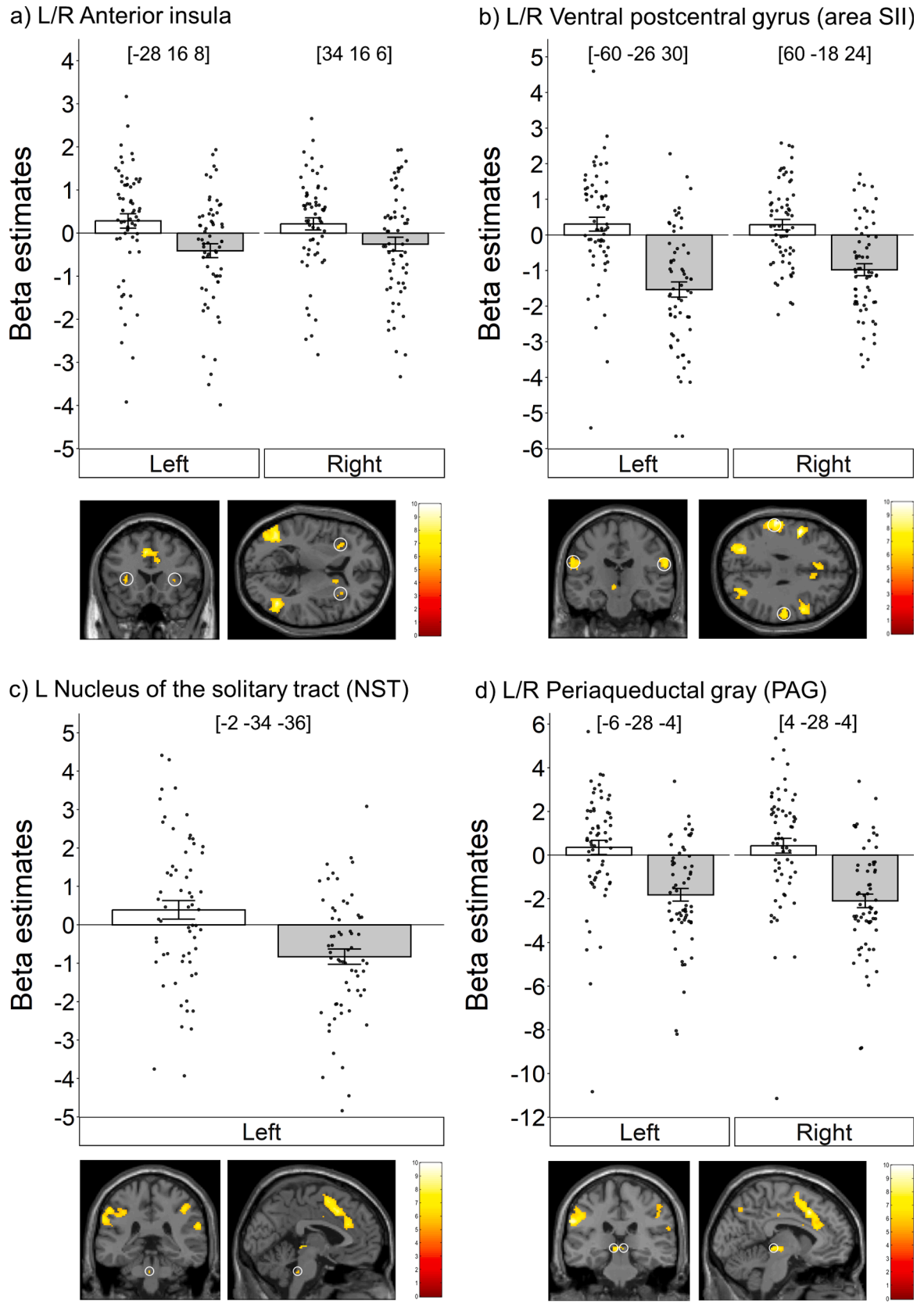
We found no significant correlation between activation in the two amygdala ROIs and sIgA secretion rate (see Table S3 for all correlation coefficients).

3.3.5. Correlation between trait disgust, perceived disease vulnerability and brain activation in key regions of the neuroimmune axis during perception of the contagion videos (ROI-based analysis)

We found that trait *Contamination Disgust*, which was determined by the Disgust Scale (Olatunji et al., 2007) prior to the neuroimaging session, positively correlated with activation in both the left (r = 0.32, p = 0.012) and the right amygdala ROIs (r = 0.27, p = 0.033) in the contrast of *Contagion* > *Healthy Human* (see Fig. 4b). *Perceived Infectability*, which had been determined before scanning, also positively correlated with activation in the right amygdala (r = 0.27, p = 0.034). In contrast, activation in the two insula ROIs was not correlated with any of the trait personality measures (see SI Appendix, Supplementary Table S3).

4. Discussion

The present study assessed the neural underpinnings of the perception of contagion cues that are typical for respiratory viral infections. For this, we presented videos showing either contagious or non-contagious situations from everyday life and measured consecutive brain activation and sIgA release in saliva as an indicator of the mucosal immune response. We found that the perception of sneezing and sick persons was accompanied by increased activation in the anterior insula and other brain regions that are either part of the neuroimmune axis or are involved in the (somatosensory) representation of the respiratory tract. Contagion cue-related activation in the anterior insula further positively correlated with the general increase of sIgA secretion during the experiment and the contagion and disgust ratings of the videos. This



(caption on next page)

Fig. 3. Brain activation in the *Contagion* > *Healthy Human* contrast. Figure depicts renderings of activation on coronal and horizontal (a & b) or coronal and axial slices (c & d) (at $p < 0.05$, FWE-corrected at peak) and displays plots of mean values and standard errors of parameter estimates as well as real data points from the contrasts of *Contagion* > *implicit baseline* (white) and *Healthy Human* > *implicit baseline* (gray). (a) Cluster maxima in the left and right anterior insula, (b) cluster maxima in the left and right ventral postcentral gyrus (area SII), (c) cluster maximum in the NST, (d) cluster maxima in the PAG. For illustration purposes, parameter estimates were extracted with Marsbar from spherical volumes centered around the coordinates of the respective activation maxima (see also Table S1). The spheres in the left and right anterior insula and the postcentral gyrus had a radius of 10 mm, while those in the NST and PAG had a radius of 4 mm to account for the relatively smaller size of the clusters.

Table 1
Brain activation in the anterior insula and amygdala during video perception ($p < 0.05$, FWE-corrected at peak level, no clustersize restriction). T-values and FWE-corrected p-values are shown in parenthesis.

Brain region	Contagion >Healthy Human	Contagion >Low-Level	Healthy Human >Low-Level
L/R anterior insula	−28 16 8 [6.44, 0.002] 32 22 4 [5.91, 0.035]	−30 20 6 [6.04, 0.006] 30 22 2 [5.56, 0.031]	n.s.
L/R amygdala	n.s.	18–4 −16 [6.88, <0.001]	−16–8 −14 [5.48, 0.042] 18–6 −16 [5.73, 0.001]

implies a central immunomodulatory role of this brain region that may prepare the oral mucosae for subsequent pathogen contact. In contrast, the amygdala showed heightened activation to all videos displaying humans, regardless of contagion signs, which might reflect nonspecific alertness to humans as being potentially contagious regardless of visible sickness signs. This may also be underscored by the observed correlations between increased amygdala activation and general disgust sensitivity. In that way, our data may also suggest a functional dissociation of the anterior insula and amygdala in response to respiratory contagion cues. While the anterior insula only responded to the unequivocal predictors of an immunological challenge (i.e., typical signs of an imminent contagion threat) and was related to the proactive release of sIgA, the amygdala appeared to be more engaged by the salience and disgust-eliciting potential of possible contagion threats, i.e., both healthy and sick humans. In this manner, these two key regions of the neuroimmune axis make slightly different contributions to the cognitive and physiological mechanisms that protect humans against respiratory infection.

As a central part of the neuroimmune axis, the insula orchestrates immune responses to sensory cues from different modalities and contributes to several defensive physiological mechanisms such as heightened inflammation and activation of immune cells to ward off acute pathogen infestation (Schiller et al., 2021). Its intricate connections with neuroimmune pathways thereby not only contribute to the ability to combat infection, but may also mediate responses to predictors of upcoming immunological challenge, which has been repeatedly observed in rodent models (Koren et al., 2021; Ramírez-Amaya and Bermúdez-Rattoni, 1999). The present findings extend these observations by showing that the human anterior insula responded to typical predictors of imminent respiratory contagion that cannot be easily avoided in real life (e.g., sneezing persons or persons with characteristic symptoms of a cold in the near vicinity), which was accompanied by a proactive increase of antibody release in saliva as well as enhanced ratings of disgust and contagion potential. These observations are consistent with the idea of the insula as a central "interoceptive hub", that not only integrates sensory information from different modalities, but connects it with cognitions and emotions that can then be used to predict future states of the body. As a consequence, this "interoceptive inference" may enable proactive immunological or behavioral responses to protect against infection even before actual pathogen exposure (Schiller et al., 2021; see also Bhat et al., 2021; Paulus et al., 2019 for theoretical background),

Table 2
Parametric modulation of activation in the anterior insula and the amygdala during video rating ($p < 0.05$, FWE-corrected at peak level, no clustersize restriction). T-values and FWE-corrected p-values are shown in parenthesis.

Brain region	Contagion Rating	Disgust Rating
L/R anterior insula	−28 22–8 [6.65, 0.001] 32 20–6 [5.92, 0.009]	−34 18 2 [7.86, <0.001] 44 20–8 [7.65, <0.001]
L/R amygdala	n.s.	−26 8–22 [6.45, 0.001]

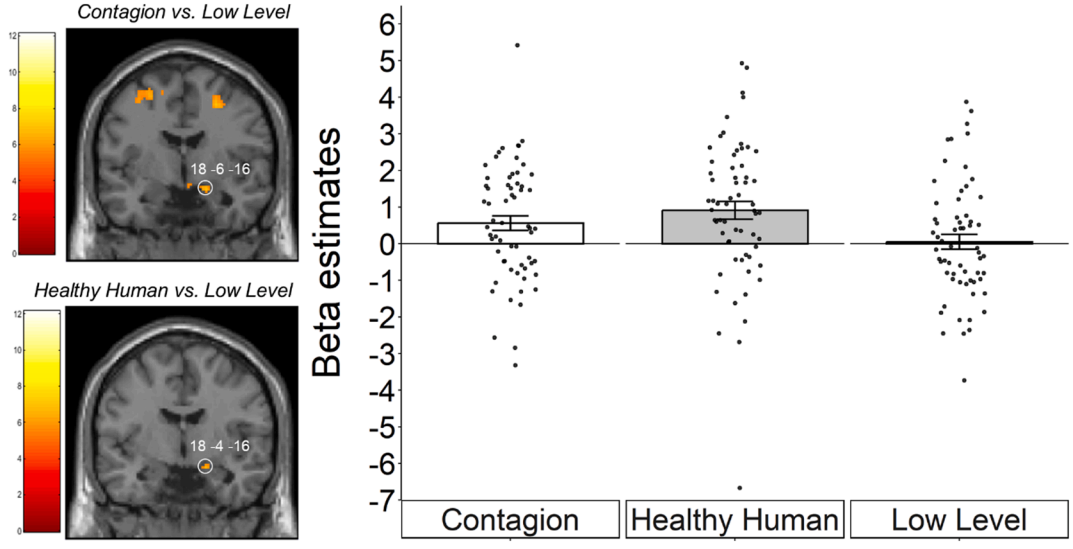


Fig. 4. Brain activation in the amygdala during perception of the different video conditions. Figure depicts renderings of activation on coronal slices (at $p < 0.05$, FWE-corrected at peak) and displays plots of mean values and standard errors of parameter estimates as well as real data points from the three contrasts against implicit baseline. Parameter estimates were extracted from a sphere at 18–6 −16 (radius = 10 mm).

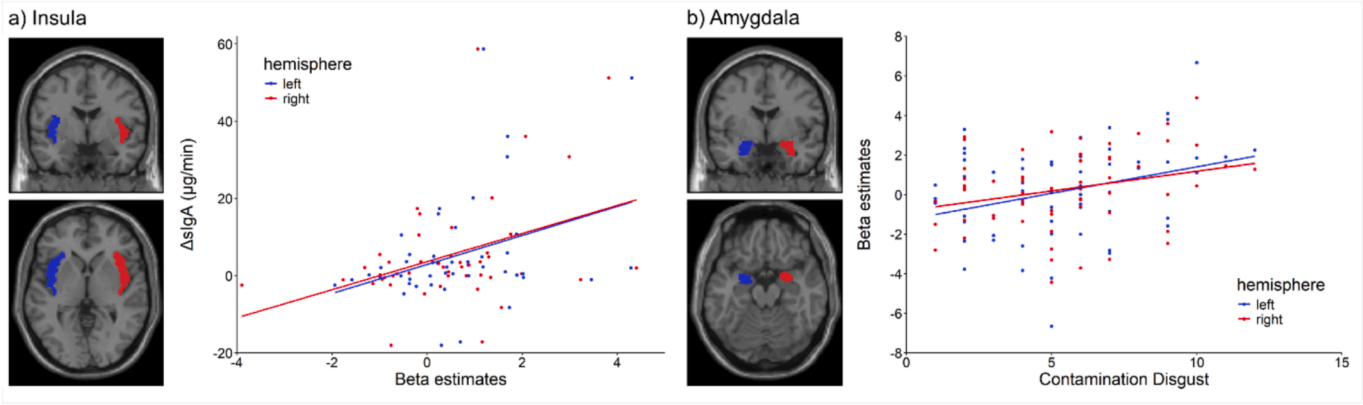


Fig. 5. Correlations between beta estimates of the contrast *Contagion* > *Healthy Human* from the anatomical ROIs of (a) the bilateral insula and sIgA secretion, and (b) the bilateral amygdala and trait contamination disgust. Correlations were significant at $p < 0.05$, two-tailed.

like when retrieving conditioned immune responses (Koren et al., 2021; Ramírez-Amaya and Bermúdez-Rattoni, 1999). This further seems plausible given the anatomical connections of the insula with the NST and PAG, two regions that were also activated during perception of the contagion videos. Through the NST, the insula receives ascending interoceptive information from the vagus nerve, and then transmits salience-related information to the PAG and other regions of the salience network. The PAG itself projects back to autonomic efferent nuclei and the vagus nerve, thus closing the circuit of NST, insula and PAG (Molnar-Szakacs and Uddin, 2022), which enables the insula to exert control over defensive behavioral and immune responses. In addition to that, previous studies also linked the PAG to automatic breath-holding in response to imminent threats, especially if threats were beyond one's control (Faull et al., 2019), while the NST was found to be involved in oral-pharyngeal reflexes (Miller, 2002). One may speculate that their coactivation with the anterior insula could have also contributed to breath-holding as a defensive behavioral response, that – in a real-world situation – would have prevented deep aspiration of the aerosols and droplets emitted by sneezing. Simultaneously increased activation in the secondary somatosensory cortex (area SII in the ventral postcentral gyrus) may further support this assumption. Previous studies found activation in area SII alongside the insula during interoceptive awareness of strong prickle sensations in the nose (Langer et al., 2010), tactile stimulation of the nasal mucosae (Gastl et al., 2014), and oral somatosensory awareness (Haggard and de Boer, 2014). Area SII has also been observed to contribute to internally guided changes in respiration rate during both the execution, observation, and mental imagery of actions that require significant alterations in breathing (Pellicano et al., 2021). This suggests that increased somatosensory activation may have either contributed to the internal representation of watching someone sneeze, or it may have also been involved in changing the breathing pattern. Alongside the anterior insula, PAG, NST, it thus could have been involved in the process of "interoceptive inference", which enabled proactive defensive responses in the respiratory tract (e.g., increased antibody release at the oral mucosae or breath holding) even without actual pathogen contact.

In contrast to the anterior insula, the amygdala was not specifically activated by contagion cues, but responded to videos displaying humans in general, a finding that to some extent defied our expectation regarding its established role in the neuroimmune axis (Doenlen et al., 2011; Harrison et al., 2009b, 2009a; Hayes et al., 2014; Kipps et al., 2007; Lekander et al., 2016; Schäfer et al., 2005). Since the amygdala is also central in fear and arousal, it is particularly responsive to salient and threatening stimuli in the environment (Amorapanth et al., 2000; Williams et al., 2005). Our finding thus implies that all humans or the human faces shown in the videos may have been perceived as particularly salient. Taking a step further, in contagious contexts, where encounters with infected conspecifics are very likely and asymptomatic individuals can transmit respiratory diseases even by simply breathing or speaking, all humans and especially human faces – regardless of sickness signs – may be perceived as a contagion threat, which could explain the indiscriminate activation of the amygdala. During the COVID-19 pandemic, behavioral experiments showed that people exhibited increased social avoidance of all humans even in the absence of disease indicators (e.g., Diekhof et al., 2024; Holt et al., 2022). Further adding to this, in the present study amygdala activation scaled with higher disgust ratings, while increased trait contamination disgust and disease vulnerability predisposed individuals for a higher amygdala activation in response to the contagion videos. It may thus be plausible to assume that our experimental setting, which clearly focused on respiratory contagion risk, since one third of the videos showed humans carrying explicit contagion signs, may have led to a nonspecific alertness to humans in general or could have even promoted a threat interpretation bias in the amygdala (Leathers-Smith and Davey, 2011). This assumption would also be supported by the finding that the healthy humans displayed in the videos were also rated as significantly more contagious than the *Low-Level* videos. Altogether, amygdala activation thus resembled that of a general threat detector in social situations, without showing any specific association with the proactive mucosal immune response. It has been further suggested that the amygdala may be particularly responsive to human faces, especially when carrying atypical features (Todorov, 2012). We therefore propose that in the present study the amygdala could have been predominantly engaged in cognitive processes that involved the heightened processing of human faces for possible contagion signs (e.g., sneezes, paleness) that represent aberrant features from the visual and behavioral norm.

Apart from that, we also found increased activation in a network of frontoparietal regions (e.g., IFJ, cingulate cortex, inferior parietal lobule, and angular gyrus) that accompanied the perception and evaluation of contagious situations, but was neither engaged during watching healthy humans nor during the evaluation of disgust. The anterior part of the insula is primarily connected to the frontoparietal attention network and may thus also contribute to evaluative and higher-cognitive processes of goal-directed behavior (Katsumi et al., 2022; Royer et al., 2020; Zhao et al., 2023). Increased frontoparietal activation could have thus reflected the detection and increased attentional processing of the explicit signs of heightened infectiousness (Vossel et al., 2014).

To our knowledge, the present fMRI study is the first that explicitly assessed neural responses to respiratory contagion cues, yet it is not the first one to examine the perception of malaise indicators. Two previous fMRI studies already assessed neural activation during the perception of static faces (and odors) of persons being in an acute inflammatory state following endotoxin challenge (Leschak et al., 2022; Regenbogen et al.,

2017). The first study (Regenbogen et al., 2017) found increased activation in the posterior insula alongside a network of somatosensory and frontoparietal regions for attentional control. In contrast, the second study (Leschak et al., 2022) failed to observe differential activation in the insula and the amygdala, despite using similar stimuli as the first study and a region-of-interest approach. Instead, they found reduced activation in the ventromedial frontal cortex with decreased likability judgements. Several important differences may explain discrepant findings: First and foremost, our study assessed responses to a selection of respiratory contagion videos showing sick or sneezing persons in everyday situations. This not only differs significantly from the static facial displays used by the other two studies, but the animated stimuli from our study also unequivocally indicated the actual contagion threat inherent to the (in)visibly spreading aerosols that are difficult to avoid. Second, by focusing on the contagion and disgust potential of the videos, our participants were already primed towards a potentially contagious context, which was further enforced by the nature of the videos themselves. In contrast, the other two studies explicitly avoided this contagion association, but asked for likability judgements instead, which probably moved the focus towards the social reward value of the sick faces rather than their contagion potential. Since neither of the two previous studies examined the physiological immune response of their participants, it remains undetermined whether the rather subtle facial inflammation indicators activated any defensive immune reactions at all. Unfortunately, this also precludes inferences about neuro-immune-interactions evoked by their specific class of malaise cues.

Finally, the results of our study may be to some extent limited. Although the event-related fMRI design was optimized for dissociating brain activation elicited by contagious from non-contagions human displays as well as from matched background settings, we were unable to avoid carryover effects between conditions. This may not only explain the involvement of the amygdala in both conditions that included humans, indicating their increased salience overall, but may also limit the interpretation of the rise in sIgA from its initial baseline, that was collected directly after the anatomical scan, to the two post-stimulation samples, taken after each functional scan. Therefore, our design did not allow for an unequivocal assignment of the sIgA rise to the contagion condition alone, since participants underwent stimulation by all three conditions in between samples. This was different from our previous approaches (Keller et al., 2024, 2023, 2022; Keller and Diekhof, 2024), in which we had strictly separated the contagion-related from the control stimulation, either through different test appointments or by testing independent groups. Nevertheless, we think that the general stress potential of the confined and noisy scanner-environment did not significantly influence sIgA release during the functional scans. The sIgA baseline sample was collected after the localizer and anatomical scan (after ~ 9 min in the scanner), during which participants already had had time to adjust to the situation in the MR scanner. Any increases in sIgA during the functional scans were therefore most likely related to the contagion condition, and maybe also in part to the false assignment of some healthy humans as potentially contagious. This would also be consistent with one of our previous studies that found a tendency for misattribution of the healthy human control condition as potentially contagious in an experimental setting that was associated with the pandemic (see Supplement of Keller et al., 2023).

In conclusion, our data indicate that the perception of animated respiratory contagion cues activates the anterior insula and other brain regions implicated in the neuroimmune-axis and the somatosensory representation of the respiratory tract. This neuroimmune axis may thereby initiate both proactive immunological and defensive cognitive responses, such as disgust and contagion awareness, and supposedly reflects the coordination of the PIS and the BIS to reduce infection probability associated with an unavoidable contagion threat. Notably, the simultaneous activation of neural and immunological responses already occurred in the presence of potential disease indicators. We therefore propose that prior experience with similar contagious situations could trigger the associated neuro-immune cascade to prepare for the forthcoming immunological challenge, akin to immune conditioning, retrieval of immune memories, or even "interoceptive inference".

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used the tool *UHHGBT* (https://uhhgpt.uni-hamburg.de) in order to improve readability and language of a few sections of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Author contributions

E.K.D acquired funding, conceptualized and designed the study, and supervised the project. J.K. contributed to the design of the study, programmed the experiment, performed data acquisition and formal analysis. E.K.D. reviewed the results, and both J.K. and E.K.D. wrote the manuscript.

Significance statement

Respiratory pathogens emitted by sneezing, talking or breathing are difficult to avoid and pose a constant threat in human social groups. This study delves into the neural mechanism evoked by contagion cues typically associated with respiratory infections, and sheds light on its relation with the proactive mucosal immune response humans engage to mitigate potential pathogen exposure. By investigating the brain's reactions to stimuli depicting respiratory symptoms, the research elucidates the pivotal role of the anterior insula in orchestrating early immunomodulatory responses at the oral mucosae that are complemented by the amygdala's vigilance towards potential contagion risks. Both regions may thereby be critical components of a neuro-immune axis that helps humans to prepare adaptive countermeasures against unavoidable contagion threats.

CRediT authorship contribution statement

Judith K. Keller: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Esther K. Diekhof:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2025.01.016.

Data availability

Data will be made available on request.

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2.4.1 Supplementary material and methods of Chapter IV

SI Appendix

of

Visual Cues of Respiratory Contagion: Their Impact on Neuroimmune Activation and Mucosal Immune Responses in Humans

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1. Supplementary Results:

Table S1: Brain activation during video perception (p<.05, corrected for family-wise error). T-contrasts with coordinates and t-values in parenthesis. The strongest maxima in a given cluster are marked in bold for the left and right hemisphere. For these, we also report not only the t-value, but also the peak-level FWE-corrected p-value and clustersize (k) in parenthesis.

Brain region	Contagion > Healthy Human	Contagion > Low- Level	Healthy Human > Low-Level
a) Key regions of the neuro-immune-axis			
L/R anterior insula	**-28 16 8 [6.44, .002, 50]**	**-30 20 6 [6.04, .006, 25]**	n.s.
	32 22 4 [5.91, .010, 9]	**30 22 2 [5.56, .031, 2]**	
	34 16 6 [5.43]		
L/R amygdala	n.s.		**-16 -8 -14 [5.48, .042, 1]**
		18 -4 -16 [6.88, <.001, 22]	**18 -6 -16 [5.73, .001, 10]**
b) Additional regions activated in both contagion contrasts			
L/R postcentral gyrus / supramarginal gyrus	**-60 -26 30 [9.65, <.001, 2580]**	**-52 -42 24 [6.52, .001, 121]**	n.s.
	-54 -32 44 [8.07]	-50 -44 34 [5.95]	
	-58 -36 30 [7.05]		
	-42 -40 40 [6.92]		
	-38 -42 50 [6.89]		
	60 -18 24 [7.64, <.001, 198]	**56 -40 22 [6.76, .001, 373]**	
	60 -14 32 [7.49]		
	62 -34 24 [6.92]		
	42 -36 48 [6.96]		
	34 -44 50 [6.72]		
	50 -30 52 [6.44]		
L/R periaquaeductal gray (PAG)	**-6 -28 -4 [7.64, <.001, 84]**	**-12 -22 -10 [6.99, <.001, 67]**	n.s.
	-12 -20 -8 [5.97]	-6 -28 -6 [6.7]	
		-6 -18 -2 [5.95]	
	4 -28 -4 [6.15, .004, 84]	**12 -26 -8 [6.28, .003, 37]**	
		6 -8 12 [5.87]	
		6 -22 -6 [5.86]	
		4 -2 -12 [5.72]	
		12 -12 -4 [5.63]	
		24 -22 -8 [5.47]	
L/R nucleus of the solitary tract (NST)	**-2 -34 -36 [5.73, .018, 9]**	**2 -36 -36 [6.10, .005, 10]**	n.s.
L/R inferior frontal junction (IFJ precentral sulcus/ inferior frontal sulcus)	**-48 4 30 [9.95, <.001, 481]**	**-40 28 24 [6.82, <.001, 132]**	n.s.
	-54 10 14 [7.92]	-46 18 26 [6.03]	
		-34 16 30 [5.7]	
	46 8 18 [7.93, <.001, 2478]	**44 16 26 [7.42, <.001, 619]**	
	54 8 30 [7.9]	42 30 32 [5.61]	

Continuation of Table S1.

Brain region	Contagion > Healthy Human	Contagion > Low- Level	Healthy Human > Low-Level
b) Additional regions activated in both contagion contrasts			
L/R superior frontal gyrus/ sulcus	**-20 2 60 [9.05, <.001, 2478]**	**-28 54 10 [6.02, .007, 22]**	n.s.
	-26 -6 54 [7.96]	-6 54 22 [5.76]	
	28 -4 56 [7.79, <.001, 2478]		
L/R pre-supplementary motor area	**-6 18 42 [9.53, <.001, 2478]**	**-4 16 44 [8.11, <.001, 1474]**	n.s.
	6 26 36 [5.89, <.001, 2478]	**4 20 40 [6.48, .001, 1474]**	
L/R anterior cingulate gyrus		**-8 24 34 [8.21, <.001, 1474]**	n.s.
		-8 32 28 [7.35]	
	8 40 22 [7.03, <.001, 35]		
L/R precuneus	**-10 -54 56 [5.51, .037, 1]**	**-4 -68 44 [6.47, .001, 156]**	n.s.
		12 -70 46 [5.68, .021, 8]	
		6 -66 32 [5.62]	
L/R caudate nucleus		**-12 6 12 [7.5, <.001, 84]**	n.s.
	14 14 4 [6.61, .001, 27]	**12 4 8 [5.58, .029, 6]**	
		16 14 4 [6.37]	
L/R inferior occipital gyrus/ middle temporal cortex	**-44 -64 4 [8.94, <.001, 693]**	**-48 -62 8 [7.56, <.001, 322]**	n.s.
	-42 -70 -2 [8.4]	-38 -58 12 [7.54]	
	-42 -76 6 [7.59]	-56 -50 14 [6.54]	
	-28 -88 0 [5.62]		
	44 -62 6 [9.22, <.001, 591]	**44 -62 4 [7.77, <.001, 1891]**	
	50 -58 -2 [8.29]	52 -44 10 [7.23]	
	48 -70 2 [7.01]	62 -42 12 [7.19]	
c) Additional regions only activated in the Contagion > Healthy Human contrast			
L/R middle frontal gyrus	**-32 42 20 [6.13, .005, 12]**	n.s.	n.s.
	-42 42 20 [5.47]		
	38 44 22 [5.54, .034, 1]		
L/R inferior frontal sulcus	**-38 36 14 [7.41, <.001, 188]**	n.s.	n.s.
	-38 26 22 [6.46]		
	-46 32 16 [5.87]		
	48 36 10 [6.54, .001, 51]		
	38 32 12 [5.57]		
L/R inferior parietal lobule	**-28 -46 48 [9.04, <.001, 2580]**	n.s.	n.s.
	22 -66 40 [6.95, <.001, 365]		
	18 -72 46 [6.35]		
	24 -56 48 [5.42]		

Continuation of Table S1.

Brain region	Contagion > Healthy Human	Contagion > Low- Level	Healthy Human > Low-Level
c) Additional regions only activated in the Contagion > Healthy Human contrast			
L posterior cingulate gyrus	**-10 -24 40 [6.02, .007, 16]**	**0 -18 38 [6.10, .005, 13]**	n.s.
	-4 -2 36 [5.58]		
L/R superior occipital cortex	**-26 -74 34 [9.19, <.001, 2580]**	n.s.	n.s.
L/R cerebellum	**-28 -56 -30 [6.68, .001, 46]**	n.s.	n.s.
	36 -54 -30 [7.23, <.001, 134]		
	34 -40 -36 [6.63]		
	28 -62 -26 [6.6]		
	8 -68 -26 [5.97]		
d) Remaining activations			
L posterior orbitofrontal gyrus	n.s.	**-34 18 -18 [6.31, .003, 10]**	n.s.
L/R precentral gyrus	n.s.	**-42 0 38 [6.83, <.001, 33]**	n.s.
		-58 8 18 [6.36]	
		-54 4 34 [5.6]	
		50 4 48 [6.46, .002, 619]	
L angular gyrus/ superior parietal lobule	n.s.	**-38 -56 48 [6.16, .004, 48]**	n.s.
		-16 -66 58 [5.82]	
		-34 -46 44 [5.72]	
L/R calcarine fissure/ inferior occipital cortex	n.s.	**-34 -86 -10 [10.97, <.001, 1400]**	**-22 -92 -12 [10.35, <.001, 507]**
		-26 -96 -2 [9.79]	-16 -98 -10 [9.49]
		-40 -78 -16 [8.9]	-34 -88 -12 [9.9]
		22 -96 0 [11.37, <.001, 1891]	**28 -94 -8 [12.11, <.001, 448]**
		44 -78 -12 [10.64]	20 -98 -4 [10.79]
		34 -90 -6 [10.5]	40 -84 -12 [9.86]
		40 -82 -10 [10.24]	
L/R fusiform face area	**-44 -44 -20 [6.78, <.001, 23]**	**-40 -46 -22 [10.52, <.001, 1400]**	**-40 -46 -24 [7.33, <.001, 44]**
	50 -44 -20 [6.23, .003, 7]	**44 -50 -22 [12.09, <.001, 1891]**	**42 -48 -24 [7.39, <.001, 141]**
	46 -54 -18 [5.94]		

Table S2: Parametric modulation of brain activation during video rating (p<.05, corrected for family-wise error). T-contrasts (parametric vector against implicit baseline) with coordinates and t-values in parenthesis. The strongest maxima in a cluster are marked in bold for each hemisphere. For these, we also report not only the t-value, but also the peak-level FWE-corrected p-value and clustersize (k) in parenthesis.

Brain region	Contagion Rating	Disgust Rating
a) Key regions of the neuro-immune-axis		
L/R anterior insula (extending into posterior orbitofrontal cortex)	**-28 22 -8 [6.65, .001, 502]**	**-34 18 2 [7.86, <.001, 336]**
	-40 20 -4 [6.60]	-40 14 -2 [6.89]
		-42 22 -12 [6.67]
		-30 16 -18 [5.56]
	32 20 -6 [5.92, .009, 74]	**44 20 -8 [7.65, <.001, 397]**
	38 22 -4 [5.56]	34 20 2 [7.09]
	36 30 -2 [5.70]	26 14 -20 [5.69]
	42 22 -20 [6.12]	
L/R amygdala	n.s.	**-26 8 -22 [6.45, .001, 10]**
b) Additional regions found in both contrasts		
L/R supramarginal gyrus/ inferior parietal lobule	n.s.	**52 -36 56 [5.92, .009, 34]**
		46 -40 48 [5.58]
L inferior frontal junction	**-42 8 42 [6.88, <.001, 1014]**	**-40 6 46 [6.65, .001, 88]**
	-48 8 48 [6.60]	
L/R PAG	**-8 -22 -12 [5.53, .031, 1]**	n.s.
	8 -28 -6 [5.62, .023, 3]	
L/R inferior occipital gyrus/ middle temporal gyrus	**-48 -68 8 [8.25, <.001, 2705]**	**-54 -68 6 [9.05, <.001, 1669]**
	-38 -88 -12 [7.57]	-54 -54 10 [8.33]
	-54 -52 10 [8.06]	
	-50 -56 4 [7.86]	
	36 -84 -16 [8.60, <.001, 2705]	**48 -60 6 [9.20, <.001, 2440]**
	44 -60 8 [7.97]	52 -70 2 [8.54]
	-52 -56 6 [7.34]	
L/R fusiform face area	**-44 -42 -18 [7.06, <.001, 2028]**	**-42 -48 -22 [8.84, <.001, 1669]**
	42 -48 -18 [8.51, <.001, 2705]	**44 -48 -20 [8.58, <.001, 2440]**
L/R inferior frontal gyrus	**-32 56 0 [7.61, <.001, 1014]**	**-56 18 14 [6.03, .006, 39]**
	-38 42 2 [7]	-48 46 -2 [5.78]
	-50 42 0 [6.94]	
	-54 14 10 [6.64]	
	56 16 8 [6.94, <.001, 533]	**56 22 16 [6.84, <.001, 397]**
	54 22 24 [6.91]	44 12 18 [5.64]
	50 18 30 [6.71]	

Continuation of Table S2.

Brain region	Contagion Rating	Disgust Rating
b) Additional regions found in both contrasts		
L/R cingulate gyrus	**-6 22 44 [6.61, <.001, 354]**	
	-4 -22 28 [5.81]	
	8 22 40 [6.99, <.001, 354]	**2 30 30 [5.56, .030, 3]**
	0 -36 26 [5.9]	
	-20 56 30 [5.46]	**8 16 68 [6.08, .005, 17]**
L/R superior frontal gyrus	**-30 60 16 [5.63, .022, 10]**	**-12 16 66 [6.19, .004, 15]**
	-18 58 -10 [5.55]	-26 52 20 [6.15]
	-22 -6 50 [5.55]	
L/R caudate nucleus	**-12 10 4 [6.25, .003, 36]**	**-8 12 4 [5.70, .009, 15]**
	-14 2 16 [6.02]	-6 14 0 [5.73]
	12 12 8 [5.96, .008, 16]	**12 10 8 [6.42, .002, 16]**
L/R pre-supplementary motor area	**-4 26 42 [6.71, .001, 354]**	**-2 22 42 [6.28, .003, 88]**
	-2 38 34 [6.6]	
	8 22 42 [6.66, <.001, 354]	**4 22 44 [6.02, .003, 88]**
	0 14 58 [5.92]	
Superior parietal cortex	**-24 -72 56 [5.50, .034, 2]**	**40 -50 60 [6.00, .007, 16]**
c) Additional regions specific for the Contagion Rating		
L/R precuneus	**-8 -68 38 [7.25, <.001, 475]**	n.s.
	-10 -74 46 [6.6]	
	-6 -64 64 [5.53]	
	6 -70 48 [6.60, .001, 475]	
	10 -68 50 [6.65]	
L/R inferior parietal lobule/ angular gyrus	**-36 -56 50 [6.97, <.001, 757]**	n.s.
	-44 -46 46 [6.7]	
	-34 -54 42 [6.72]	
	-52 -52 38 [5.45]	
	38 -56 42 [6.98, <.001, 757]	
	40 -46 36 [6.82]	
	36 -48 48 [6.42]	
L/R middle frontal gyrus	**-36 -2 54 [5.49, .036, 2]**	n.s.
	-30 2 64 [5.73]	
	-48 38 18 [6.47]	
	40 6 58 [7.93, <.001, 188]	
	32 6 50 [6.60]	
	48 8 52 [6.09]	
	42 38 24 [6.35]	
	48 34 30 [6.23]	
	40 46 -10 [5.73]	
L medial orbitofrontal gyrus	**-20 44 -16 [6.68, .001, 18]**	n.s.

Continuation of Table S2.

Brain region	Contagion Rating	Disgust Rating
d) Additional regions specific for the Disgust Rating		
R inferior temporal gyrus	**38 -2 -42 [6.09, .005, 11]**	n.s.
	44 -22 -22 [5.48]	
L/R calcarine sulcus	n.s.	**-14 -96 -2 [7.14, <.001, 1669]**
		18 -92 2 [9.28, <.001, 2440]
R intraparietal sulcus	n.s.	**32 -46 46 [6.32]**
L/R thalamus	n.s.	**-4 -20 6 [6.15, .004, 23]**
		-6 -12 10 [5.68]
		10 -12 6 [5.97, .008, 10]

Supplementary Table S3: Correlations between beta estimates from the t-contrast *Contagion > Healthy Human*, extracted from anatomical ROIs in the insula and amygdala, and (a) sIgA secretion rates, as well as (b) subscales of the trait personality measures, i.e. scores from the *Disgust Scale* and *Perceived Vulnerability of Disease Questionnaire*. For (a) we report Spearman's *rho* with p-values in parenthesis, since sIgA secretion rates significantly deviated from a normal distribution as indicated by the Kolmogorov-Smirnov test. In case of (b) we report Pearson's r, since data were parametric. Significant correlations (p<.05, two-tailed) are highlighted in bold.

	Insula Beta estimates		*Amygdala* Beta estimates	
	Left hemisphere	Right hemisphere	Left hemisphere	Right hemisphere
a) Correlations with sIgA secretion rate (n=47)				
Baseline	.08 (.606)	.22 (.145)	.23 (.126)	.07 (.620)
Post-Stimulation 1	.17 (.249)	.29 (.050)	.07 (.631)	.14 (.334)
Post-Stimulation 2	.21 (.156)	**.39 (.008)**	.20 (.188)	.13 (.401)
Delta sIgA Overall	**.30 (.044)**	**.31 (.035)**	.01 (.970)	.17 (.242)
b) Correlations with trait personality measures (n=62)				
Contamination Disgust	.08 (.535)	.10 (.462)	**.32 (.012)**	**.27 (.033)**
Core Disgust	-.21 (.094)	-.16 (.221)	.13 (.315)	-.02 (.897)
Germ Aversion	.05 (.709)	-.01 (.963)	.08 (.531)	.01 (.959)
Perceived Infectability	.05 (.698)	.05 (.693)	.20 (.113)	**.27 (.034)**

2. Supplementary Material & Methods

Supplementary Table S4: Trial sequences of both runs with video condition shown, length of jitter 1 and jitter 2 (ms) and type of rating following the video condition.

	Run 1				***Run 2***			
Seq.	*Video condition*	*Jitter 1*	*Rating*	*Jitter 2*	*Video condition*	*Jitter 1*	*Rating*	*Jitter 2*
1	Dummy	3000	Contagion	1250	Dummy	3000	Disgust	750
2	Dummy	3500	Disgust	750	Dummy	3500	Contagion	1250
3	Contagion	1500	Disgust	500	Healthy Human	5000	Disgust	1250
4	Healthy Human	1500	Contagion	750	Low-Level	2500	Contagion	1250
5	Low-Level	4000	Disgust	500	Contagion	4000	Disgust	500
6	Healthy Human	2000	Contagion	750	Low-Level	1500	Contagion	1000
7	Low-Level	2000	Contagion	500	Healthy Human	4500	Disgust	750
8	Contagion	2000	Disgust	1000	Contagion	3500	Contagion	1000
9	Contagion	1500	Contagion	750	Contagion	5000	Contagion	1250
10	Low-Level	2500	Contagion	1000	Low-Level	4000	Contagion	750
11	Healthy Human	2500	Disgust	1250	Healthy Human	4000	Disgust	1000
12	Healthy Human	3000	Disgust	1000	Low-Level	3500	Disgust	500
13	Contagion	3500	Disgust	500	Contagion	4500	Disgust	1250
14	Low-Level	3000	Contagion	1250	Healthy Human	2500	Contagion	500
15	Healthy Human	3500	Disgust	500	Contagion	2000	Disgust	750
16	Low-Level	4500	Disgust	500	Low-Level	3000	Disgust	1250
17	Contagion	3000	Disgust	1250	Low-Level	4000	Contagion	1000
18	Low-Level	4000	Contagion	750	Healthy Human	1500	Disgust	1250
19	Low-Level	3500	Contagion	1000	Contagion	2500	Contagion	500
20	Healthy Human	4000	Contagion	750	Low-Level	2000	Disgust	750
21	Contagion	5000	Disgust	1250	Healthy Human	2500	Contagion	1000
22	Healthy Human	1500	Contagion	1000	Contagion	3000	Contagion	500
23	Low-Level	5000	Disgust	1250	Contagion	1500	Contagion	1000
24	Contagion	4500	Contagion	1000	Healthy Human	2000	Contagion	750
25	Healthy Human	5000	Disgust	1250	Low-Level	1500	Disgust	500
26	Contagion	3500	Contagion	750	Healthy Human	3000	Disgust	750
27	Healthy Human	2000	Disgust	1250	Healthy Human	4500	Contagion	500
28	Low-Level	5000	Disgust	1000	Contagion	5000	Disgust	1250
29	Contagion	2000	Contagion	1250	Low-Level	4500	Disgust	500
30	Healthy Human	3000	Contagion	1000	Contagion	3500	Contagion	1000
31	Healthy Human	4000	Disgust	750	Healthy Human	2000	Disgust	750
32	Contagion	4500	Contagion	500	Healthy Human	3500	Disgust	1000
33	Low-Level	2000	Disgust	1000	Low-Level	3500	Contagion	750
34	Low-Level	1500	Disgust	750	Contagion	2500	Disgust	500
35	Healthy Human	5000	Contagion	500	Healthy Human	1500	Contagion	1250
36	Contagion	4000	Contagion	750	Contagion	1500	Disgust	750
37	Contagion	5000	Disgust	1000	Contagion	4500	Contagion	1250
38	Low-Level	2500	Contagion	500	Low-Level	2500	Disgust	1000
39	Low-Level	1500	Contagion	1250	Healthy Human	5000	Contagion	500

Continuation of Supplementary Table S4

	Run 1				***Run 2***			
Seq.	*Condition*	*Jitter 1*	*Rating*	*Jitter 2*	*Condition*	*Jitter 1*	*Rating*	*Jitter 2*
40	Healthy Human	4500	Disgust	750	Low-Level	4500	Contagion	1250
41	Contagion	2500	Contagion	1250	Contagion	4000	Disgust	500
42	Healthy Human	4500	Contagion	1000	Low-Level	5000	Disgust	1250
43	Low-Level	2000	Disgust	1250	Contagion	3000	Contagion	1000
44	Contagion	4000	Disgust	1000	Healthy Human	4000	Contagion	750
45	Contagion	2500	Contagion	750	Healthy Human	3000	Contagion	1000
46	Healthy Human	3500	Contagion	1250	Low-Level	5000	Disgust	750
47	Healthy Human	2500	Disgust	500	Low-Level	3000	Contagion	1000
48	Low-Level	3500	Contagion	750	Contagion	2000	Disgust	750
49	Low-Level	4500	Disgust	500	Healthy Human	3500	Disgust	1250
50	Contagion	3000	Disgust	500	Low-Level	2000	Contagion3	500

Supplementary Table S5: Results of the Kolmogorov-Smirnov-Test for all *variables* used in the data analyses with *IBM-SPSS* with the test statistic k, the number of cases (N) and the p-values.

	k	N	p
a) Trait measures			
Contamination Disgust	0.11	62	.169
Core Disgust	0.09	62	.200*
Germ Aversion	0.10	62	.200*
Perceived Infectability	0.12	62	.069
b) sIgA Secretion			
Baseline	0.22	47	<.001
Post-Stimulation 1	0.30	47	<.001
Post-Stimulation 2	0.29	47	<.001
Delta sIgA Overall	0.27	47	<.001
c) Beta estimates of ROIs			
Left Amygdala	0.09	62	.200*
Right Amygdala	0.05	62	.200*
Left Insula	0.09	62	.200*
Right Insula	0.10	62	.200*
d) Contagion Rating			
Contagion	0.09	62	.200*
Healthy Human	0.26	62	<.001
Low Level	0.30	62	<.001
e) Disgust Rating			
Contagion	0.07	62	.200*
Healthy Human	0.32	62	<.001
Low Level	0.30	62	<.001

*. This is a lower bound of the true significance.

3. Discussion

A concept of the reactive immune system is widely accepted, and the system is highly effective for individuals in the ongoing arms race between pathogens and hosts. However, based on its high energy costs and the restrictions an individual has once in contact with a pathogen (Hart and Hart, 2019; McDade, 2003; Yatim and Lakkis, 2015), a proactive immune system that helps avoid and prepare for potential pathogen contact is feasible. In the following, I will discuss the evidence found for a proactive physiological immune response in healthy adults, based on the findings in this thesis. Further, I will discuss the implications of the findings for future research in immunosuppressed patients. Lastly, I will focus on the origin of the found mechanisms.

3.1 The proactive physiological immune response in healthy adults

We consistently found evidence of a proactive physiological immune response in sIgA in saliva throughout all four chapters of this thesis. In each of the four studies (*see Chapters I-IV*), visual stimuli related to respiratory disease cues were utilized, resulting in increased sIgA secretion into saliva compared to baseline values. These findings are based on data from young (ages 18-35 years) and healthy individuals (no previous chronic, immunosuppressive, or psychological diseases/disorders). These data sets establish a foundation for understanding proactive immune responses and may serve as a basis for studies investigating these mechanisms in patients and for research aimed at understanding the evolutionary background.

3.1.1 Specificity of proactive physiological immune response

In *Chapter I* (Keller et al., 2022), we utilized four sets of videos varying in contagion and disgust potential. All three disease and disgust-related videos elicited a significant increase in sIgA; however, the strength of the increase, while not significant, seemed to vary. So, did participants presented with the concealed contagion video have an increased sIgA secretion of an average of 100.63%. In comparison, the aerosol video elicited an increase of 83.15%, and the core disgust video only increased the secretion by 44.79%. Previous studies showing

pictures with disgust content akin to our core disgust video could not find an increase in sIgA (Stevenson et al., 2012, 2011b). While we may have improved on the stimuli by, for example, using videos instead of pictures, one might also speculate if certain stimuli elicit certain immunomarkers more or less. The picture stimuli of the previous study were able to elicit a response in TNF-α and Albumin (Stevenson et al., 2012, 2011b) and IL-6 in blood (Schaller et al., 2010). In *Chapter II*, we found that while total sIgA increased in reaction to our respiratory contagion videos, there was a certain compartmentalization in the response, as only Sars-CoV-2 spike-specific sIgA increased, but not Sars-CoV-2 RBD-specific sIgA (*Chapter II;* Keller et al., 2023). Therefore, the proactive immune response may be so specialized that only some immune markers react proactively to certain stimuli. In addition, respiratory disease stimuli are the ones humans are confronted with most frequently (Thomas and Bomar, 2025; White and Brown, 1999) and the ones that they may not be able to avoid most of the time, therefore a proactive physiological immune response towards these stimuli is most reasonable, while other kinds of pathogens may not elicit such a strong response.

Further context may play an important role (Ackerman et al., 2020), so have some participants in *Chapter I* not perceived the aerosol video as a potential contagion risk, which may be due to open sneezing also occurring in other contexts like hay fever, which are not contagious (Lambert, 2018). This is further backed by the fact that perception of contagion risk, also correlated positively with the sIgA increase (*Chapter I;* Keller et al., 2022). In the following studies, we combined the concealed contagion and aerosol video stimuli to contextualize the open sneezing stimuli better.

It is further important to note that the data collection in this thesis was conducted during the middle to the end of the COVID-19 pandemic in Germany (see Table 1 for exact dates). While we mostly tested participants during times of low incidences and with vaccines already in place (Bundesregierung, 2023; RKI, 2025) (also Figure 3), the pandemic may have changed perception of respiratory disease symptoms (Bouayed, 2022) and disgusting stimuli

(Milkowska et al., 2021b; Spangler et al., 2024), which may have had a similar effect on our results, such as the flu season, seemed to have on the results of Brown et al. 2014, where they were not able to replicate their findings in proactive sIgA secretion outside the flu season.

Table 1: Timing of the four studies with beginning and end points as well as information on the state of the pandemic in Germany: Mandates and Vaccine status (Bundesregierung, 2023) and average of new cases (RKI, 2025) at the point of data collection.

Study	*Begin*	*End*	*Mandates*	*Vaccines (y/n; number)*	*Average of new cases*
Chapter I	May 2021	October 2021	Yes	Yes, first shot	low
Chapter II	February 2022	April 2022	Yes	Yes, two shots	high
Chapter III	March 2021	September 2022*	Yes	Yes, one to two shots	Varied from low to high
Chapter IV	February 2023	August 2023	No	Yes, two + booster	Very low

*Data was collected in three blocks originally from February 2021 to August 2021, and then two further test times as the N with valid data and fitting with cycle phases was too low: from January to March 2022, and June to September 2022

Nevertheless, there was no difference in state pathogen disgust between the acute pandemic and previously collected data (Carr et al., 2022). Further, a previous COVID-19 infection only marginally affected the perceived vulnerability to disease. This leads to the speculation that situational disease threat (such as a pandemic) may be less important to the behavioral immune response than long-lasting experience (i.e., growing up in places with higher disease threat or experiencing infection throughout life more often than the norm) (Troisi et al., 2023). Within the participant recruitment for *Chapter II* (Keller et al., 2023), we mentioned the COVID-19 pandemic specifically as the study was focused on Sars-CoV-2-specific sIgA. This focus on the pandemic did not affect the increase of total sIgA after contagion-stimuli (44.29 %) compared to the other studies (*Chapter I:* 100.63 %/ 83.5 %; *Chapter III:* 49.39 % & *Chapter IV:* 52.16

%) in which we tried to stay inconspicuous about disease-context (although not completely possible due to regalements regarding participant information's) and did not mention the pandemic specifically. However, both the contagion stimuli and the control video elicited an increase in total sIgA in *Chapter II* (*see 2.2.1*). This may be due to interpretational bias, with participants expecting contagious stimuli because the recruitment was based on the pandemic (as discussed in the discussion of *Chapter II*). Lastly in *Chapter IV* (Keller and Diekhof, 2025), we were able to replicate the sIgA increase, although this study was conducted at the very end of the health mandates, which all dropped one month into data collection and we were still able to find an increased sIgA secretion of 52.16 %, between the baseline and the PostRun2 sample (even with a slightly altered procedure, mixing control and contagion videos). Therefore, we suspect that the context of the pandemic did not influence our results; to rule this out entirely, further testing completely outside of the pandemic state would be necessary.

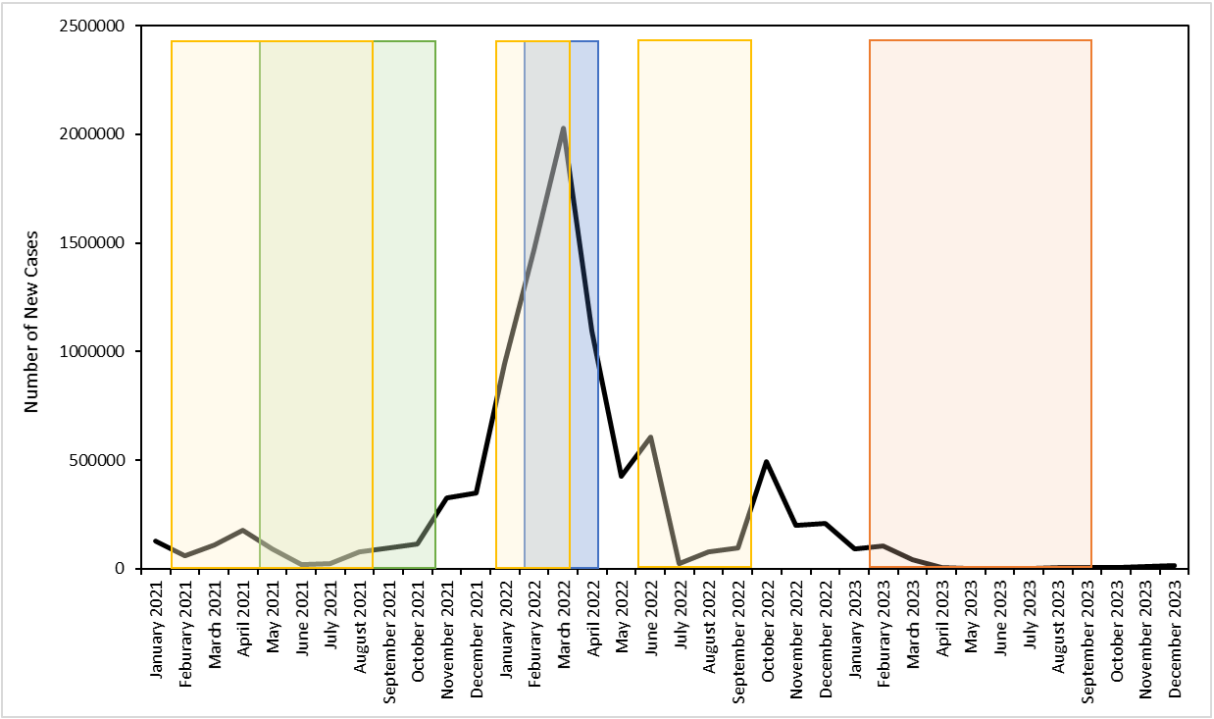

Figure 3: Number of new cases per month (between 2021 and 2023) in the age group of 18-34-year-olds (RKI, 2025). Boxes indicating timeframes of studies: *Chapter I* in green, *Chapter II* in blue, *Chapter III* in yellow and *Chapter IV* in orange.

3.1.2 Interaction with proactive behavioral immune response

The interaction between the immune system's behavioral and physiological mechanisms seems complex and difficult to entangle. Two main directions may be feasible. First, a complementary interaction of the two. After detection of a disease cue the behavioral as well as the physiological proactive immune mechanisms kick in (i.e., an individual is avoiding a sick-looking person and is also producing more immune markers). Secondly, a compensating relationship could occur, where the physiological responses kick in when the behavioral fails (i.e., avoidance not possible) or vice versa (i.e., low immune marker production). In our studies, we found evidence for a compensating relationship, with sIgA increase inversely correlating with the trait measure of contamination disgust in *Chapter I* (Keller et al., 2022). A similar correlation was found in state measures in *Chapter II*, with state disgust and respiratory interoceptive feelings correlating inversely with the Sars-CoV-2 spike protein-specific sIgA change (Keller et al., 2023). Previous studies also found similar evidence for a compensating relationship: women with a weakened reactive immune response (due to higher progesterone levels) showed a higher aversion behavior and disgust (Fleischman and Fessler, 2011) (however, we could not replicate these results in *Chapter III*, also see *3.1.3* for discussion). This seemed to also be the case for men with a weaker reactive immune response (Kandrik et al., 2017). Lastly, pVtD correlated inversely with spontaneous cytokine release and oxidative DNA damage (Gassen et al., 2018).

Nevertheless, Stevenson et al. (2015) found an increased proactive physiological immune response in sIgA and TNF-α only in participants with a high state disgust, supporting the complementary interaction theory. In addition, avoidance behavior (Miller and Maner, 2011), pVtD (Troisi et al., 2023), and disgust ratings (Spangler et al., 2024) seem to increase after recent activation of the reactive PIS (for an overview of current evidence, also see Fig. 4).

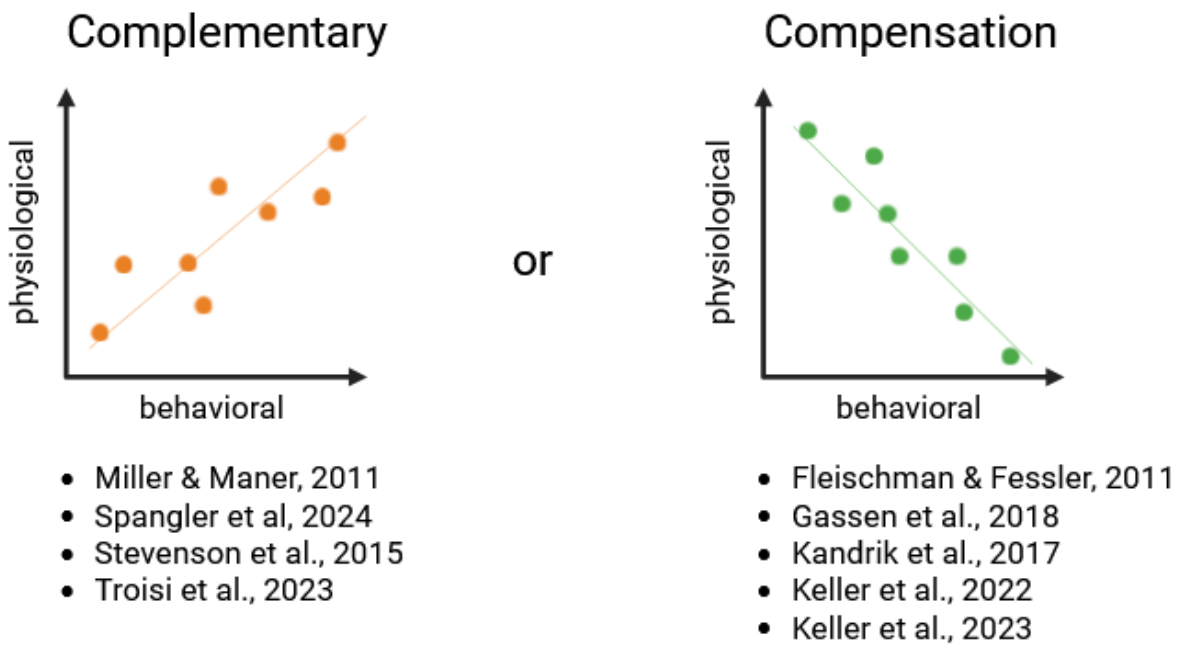

Figure 4: Hypothetical schematic and overview for evidence for the two interaction theories, Complementary on the left, Compensation on the right.

The mixed evidence may indicate a very complex system. The findings of Stevenson et al. (2015) are based on explorative analysis with a small sample size of 37 participants. We could not replicate these findings (Keller et al., 2023, 2022). Therefore, the evidence supporting the complementary interaction theory is mainly based on previous infections that alter the perception of stimuli. Miller and Maner (2011) were the first to investigate whether activation in the reactive immune system promotes activation of the BIS. They found that participants who were recently ill exhibited increased avoidance behavior towards disfigured individuals compared to healthy controls. Two further studies utilized the COVID-19 pandemic to investigate the complementary activation of the BIS after the activation of the reactive immune system. They found that people previously infected with COVID-19 had a heightened pVtD (Troisi et al., 2023) and rated even neutral stimuli as more disgusting and threatening (Spangler et al., 2024). While this suggests a situational change, the studies advocating for a compensative relationship mainly rely on relatively stable measures, such as baseline testosterone in men

(Kandrik et al., 2017), contamination disgust (Keller et al., 2022), and perceived vulnerability to disease (Gassen et al., 2018). The evidence of situational change in the complementary theory and stable measures in the compensating theory indicates that the interaction may function in both directions, depending on situational and trait variables. A possible example for the complexity is a study that found that higher progesterone (hence a lowered immune response) did not influence the disgust sensitivity, however women with a combination of higher progesterone and an acute infection showed a higher pathogen disgust compared to women with lower progesterone and an acute infection (Milkowska et al., 2019).

One may also argue that the studies showing a complementary interaction of reactive PIS activation and proactive behavioral mechanisms (Miller and Maner, 2011; Spangler et al., 2024; Troisi et al., 2023), are actually evidence for a compensating relationship, as the reactive PIS may be weakened after the recent infection (Arnold and Fuqua, 2020; Langford et al., 2020; LeVine et al., 2001; van der Sluijs et al., 2004). To grasp a better understanding of the two systems interacting, more research will be needed, including the interaction between the reactive and the proactive physiological immune system, research on participants with long-term altered immunity (e.g., autoimmune disease), measures like avoidance behavior, situational context changes, and more.

3.1.3 Influence of steroid hormones on the proactive immune response

The first to formulate the idea of the compensative function of disgust were Fleischmann & Fessler (2011), as they found that women with higher progesterone, and consequently a supposedly lower immune response (*see 1.3.1*), exhibited higher disgust ratings. The authors inferred that increased disgust may protect immunosuppressed women from coming into contact with contaminated or contagious stimuli. While this has been further found in later studies (Milkowska et al., 2021a; Żelaźniewicz et al., 2016), we could not replicate these results in *Chapter III* (Keller and Diekhof, 2024). Other studies also support our null results (Jones et

al., 2018; Milkowska et al., 2019; Olatunji et al., 2020; Stern and Shiramizu, 2022). In addition to the null results regarding the influence of female sex steroid hormones on the behavioral mechanisms, we could not find a difference between women's proactive physiological immune response in different menstrual cycle phases. This may be evidence that while progesterone seems to affect some parts of the reactive PIS, such as down regulation of inflammatory cytokines and T- as well as B-cell responses (Klein and Flanagan, 2016), it does not affect all physiological immune responses, as sIgA in saliva was neither affected in the baseline nor in the secretion change. However, this does not mean that sIgA and other immune markers are unaffected in other body regions (i.e., blood, cervical mucus).

Further, while progesterone seems not to affect the proactive immune response of sIgA in saliva, this may not be transferable to other hormones. Another study, which showed a compensating relationship between reactive PIS and behavioral immune mechanisms, used high testosterone and low cortisol as a marker for a better PIS (Kandrik et al., 2017). They found that participants with in their definition better PIS, had a lower behavioral immune response (as they did not avoid “sick looking” individuals). This study is based on the assumption that this combination of testosterone and cortisol is a marker for a better PIS on a study that showed a positive correlation between testosterone and the immune response to the hepatitis B vaccination, which seemed to be moderated by cortisol (Rantala et al., 2012). While testosterone has previously been defined as immunosuppressive (Folstad and Karter, 1992; Klein and Flanagan, 2016), mucosal sIgA seems to correlate positively with testosterone (Gettler et al., 2014; Hodges-Simeon et al., 2019). This supports the theory that the repression of some reactive mechanisms (i.e., cell-mediated immunity) may not extend to mucosal sIgA, which, in contrast, may even compensate for the downregulation of other responses (Miller and McConnell, 2012). Furthermore, estradiol, which seems to generally have immunoenhancing properties (Klein and Flanagan, 2016), has been found to be inversely correlated with sIgA in saliva (Hodges-Simeon et al., 2019) and cervical mucus (Shrier et al., 2003; Wira and Sullivan,

1985). However, we found no relationship between sIgA and estradiol at either point of measurement in our explorative analysis (*Chapter III;* Keller and Diekhof, 2024).

In general, further research on the effects of the sex steroid hormones on reactive mucosal and proactive physiological immune responses overall is needed to understand their modulating effects. It seems important to investigate the hormones in relation to each other. Our data may have been affected by the opposite effects of progesterone and estradiol in the immune system, which are both upregulated during the luteal phase compared to the follicular phase (Mesen and Young, 2015). Further, some hormones seem to moderate each other. This is not a new idea; the dual-hormone hypothesis suggests that cortisol may moderate testosterone-behavior relationships (Dekkers et al., 2019). This may also be the case for testosterone-immune correlations, as mentioned above (Rantala et al., 2012). Cortisol is also known as the stress hormone (Hellhammer et al., 2009), and research on its influence on salivary sIgA is mixed. While some studies found no relationship between the two (Cieslak et al., 2003), others found an inverse correlation (He et al., 2010; Hucklebridge et al., 1998). It seems like acute stress increases salivary sIgA for a short period, and the subsequent fall of sIgA may be correlated with the level of cortisol (Fan et al., 2009). Constant stress, on the other hand, seems to decrease sIgA in saliva (Deinzer et al., 2000). Regarding proactive behavioral mechanisms, research on hormones like cortisol and testosterone has been sparse. Recent studies found no relationship between disgust and salivary testosterone (Jones et al., 2018; Stefanczyk et al., 2024). In contrast, a study measuring testosterone in serum found a positive relationship (Kaňková et al., 2024). Cortisol has only been measured as a reaction to disgust stimuli, while two studies found no effect of disgust stimulation on cortisol (Buske-Kirschbaum et al., 2001; Hennig et al., 1996), another found a positive correlation of cortisol and disgust rating after stimulation in participants with a high disgust sensitivity (Rohrmann et al., 2004).

In the present studies, we did not factor in the extent to which our stimulation with respiratory

disease cues elicited stress and, therefore, possibly increased cortisol. However, we asked participants to evaluate their stress levels during the last two weeks and excluded participants with heightened stress, as constant stress seems to influence the baseline sIgA levels.

Research investigating the influence of hormones on the physiological proactive immune response in the future should include a wider panel of hormones to understand possible moderating effects. Further, it would be important to investigate the paradigm in a within-subject design to reduce data variability (also see *Chapter III, Limitations*).

3.1.4 Underlying brain mechanism of the Proactive immune responses

Chapter IV was the first attempt to grasp a better understanding of brain regions responsible for the detection of disease cues and the subsequent activation of proactive immune responses. We found four major regions that seemed relevant in detecting open respiratory symptoms, with the anterior insula activation correlating to the proactive sIgA increase (Keller and Diekhof, 2025).

As previously described (*see 1.4.),* we expected the anterior insula to be activated after stimulation with our respiratory disease cues, as it plays a significant role in processes related to the neuroimmune axis, such as immune conditioning (Pacheco-López et al., 2005; Ramírez-Amaya et al., 1996), storage and retrieval of immune-related information (Koren et al., 2021), and immune modulation (Hess et al., 2011). While a connectivity between the anterior insula and the other regions has previously been found: towards nucleus of the solitary tract (NST) by receiving interoceptive information from the vagus nerve and towards the periaqueductal grey (PAG) by transmitting information regarding salience to it (Molnar-Szakacs and Uddin, 2022), they have not yet been put in one system regarding the Neuro-Immune-Axis. However, the PAG has been found to be an integrative center for defensive responses such as anxiety, panic, and fear towards threats (George et al., 2019). Thus, the detection of disease cues may have elicited such a response. It has further been theorized that these negative emotions could be

integrated with the immune system via the PAG to facilitate threats (George et al., 2019), as it has been previously found to be connected to microglia (innate immune cells) during pain modulation (Doyle et al., 2017), however to what extend it interacts with the physiological proactive immune system is yet to investigated.

The NST has previously been suspected to play a role in immune regulation. It has been proposed that caudal NST neurons act as a biological rheostat, regulating peripheral inflammation via positive and negative feedback to immune cells (Jin et al., 2024). Its activation during the stimulation with respiratory disease cues may suggest that it also plays a role in regulating physiological proactive immune responses.

Lastly, as there has been no previous connection of the area SII of the somatosensory cortex and the Neuro-Immune-Axis, the activation we found may be solely based on the areas close connection to the respiratory tract ((Gastl et al., 2014; Haggard and de Boer, 2014; Langer et al., 2010; Pellicano et al., 2021), also *see Chapter IV, Discussion)*. One may speculate that the shown cues of respiratory tract infection may have led to an activation of mirror neurons, or a similar process, leading to similar interoceptive feelings. Therefore, the area SII may not be active if different disease cues were presented (i.e., vomit, skin sores, etc.). However, further research would be needed to understand if this area plays a role in increased respiratory interoceptive feelings after visual presentation of respiratory disease cues.

Further analysis will be necessary before suggesting these regions act as one system of sickness detection and proactive immune response. One option may be to perform connectivity analysis, which investigates task-related changes in the relationship between a region of interest and its connected regions (Friston et al., 1997), on our data. This may also help to grasp a better understanding of the interactions of the anterior insula and the amygdala during sickness detection and activation of the proactive immune response. Further follow-up studies are of importance. To grasp a better understanding of sIgA production elicited through the Neuro-Immune-Axis, a block design, separating the control conditions from the contagion condition,

may be more suitable. Further the use of more subtle disease cues like in Leschak et al., 2022 and Regenbogen et al., 2017; as well as other disease (i.e. skin lesions like Brown et al., 2014) and disgust (i.e. core and food disgust eliciting as in Keller et al., 2022; Stevenson et al., 2015) cues could help to disentangle which regions are activated based on the Neuro-Immune-Axis and which are only representations of the respiratory cues, which may be the case for Area SII that was found to be activated in our contagion vs. healthy human contrast and has previously connected to interoceptive representation of the upper respiratory tract (Gastl et al., 2014; Haggard and de Boer, 2014; Langer et al., 2010).

Lastly, a voxel-based morphometry analysis, which allows for comparison of concentrations of grey matter (Ashburner and Friston, 2000) and correlate these to other parameters, may be suitable. In our case, it would be interesting to correlate proactive immune responses such as disgust and contagion rating, as well as sIgA increase. This data may also be interesting when further comparing these to patients with chronic health conditions such as autoimmune diseases.

3.2 The proactive immune response in patients

Research in this thesis has been based on healthy humans to get a good understanding of how a proactive physiological immune response may work. One of the following steps would be to extend research to individuals with compromised health. Before mentioned studies have found an altering effect of acute (Milkowska et al., 2019) or recent (Miller and Maner, 2011; Spangler et al., 2024; Troisi et al., 2023) infections in otherwise healthy humans, on the proactive behavioral immune responses (e.g., avoidance, disgust). If the proactive physiological immune response also changes while the reactive immune system is temporarily compromised due to acute or recent infections (as shown by Arnold and Fuqua, 2020; Langford et al., 2020; LeVine et al., 2001; van der Sluijs et al., 2004), has not been investigated. Research on mucosal sIgA in saliva is sparse. Only one study investigated the sIgA level in tear fluid and found that baseline sIgA in tear fluid was significantly lower during upper respiratory tract infections,

while sIgA in saliva stayed unchanged (Hanstock et al., 2016). Therefore, research regarding the baseline and proactive response of sIgA during or shortly after an infection is needed. Further, while it has been attempted to test (potentially) 'immunocompromised' individuals regarding proactive behavioral immune responses, previous studies in fact solely tested either pregnant women or women in their luteal phase assuming that heightened progesterone may lead to an immunocompromised state (Fleischman and Fessler, 2011; Kaňková et al., 2024). Yet, populations of actually immunocompromised patients (e.g., patients undergoing chemotherapy for cancer treatment) have not yet been tested. Disgust in cancer and autoimmune patients has only been investigated with regard to physical examinations, such as disgust towards the sample collection process in colorectal cancer examinations (Reynolds et al., 2013) and self-perception (Schienle and Wabnegger, 2022), but not towards typical disgust-elicitors or disease-related stimuli that one may encounter in their everyday life. Further studies have yet to investigate avoidance behavior elicited by disgusting and disease-related stimuli in immunocompromised patient populations.

Findings on baseline sIgA levels of patients with autoimmune diseases in comparison to healthy controls are mixed. So, has sIgA in saliva on the one hand been found to be increased in patients with inflammatory bowel disease compared to healthy controls, while it seems to be decreased in patients with coeliac disease and Crohn's disease (Nijakowski et al., 2021; Warner et al., 1999). Patients with rheumatoid arthritis have been found to show no significant difference in sIgA levels compared to healthy controls (Chopra et al., 2012), while patients with juvenile idiopathic arthritis show lower levels of sIgA in saliva compared to healthy controls (Feres de Melo et al., 2014). In cancer patients research found that malignant tumors as well as the treatment against them decreases baseline sIgA levels (Harrison et al., 1998; Sun et al., 2016). Whether these changes in baseline mucosal immunity also influence the proactive physiological immune response to disease indicators, such as our disease videos, has yet to be investigated. A study design comparing healthy controls to immunocompromised patients may help to better

understand the interaction between the reactive and the proactive immune response. Another approach to this may be from the side of patients who deviate from normative behaviors regarding disgust and disease avoidance, i.e., patients with contamination-related obsessive-compulsive disorders (OCD). Patients with contamination-related OCD have a high contamination fear and therefore show excessive hygiene behaviors and avoidance (Olatunji et al., 2007a). While patients with severe OCD do not seem to differ in sIgA levels compared to healthy controls, other immune markers in saliva (IL-6, IL-1β, and TNF-α) were significantly increased in OCD patients (Westwell-Roper et al., 2022). Whether OCD also affects the proactive physiological immune response may be a further factor that will help understanding the interaction of the proactive behavioral and physiological mechanisms. Further, the results of such a study may be able to explain certain variance between patients and healthy controls.

3.3 Evolution vs. Learning: Where does the proactive immune system come from?

This thesis found repeated evidence for a proactive physiological immune response, and behavioral mechanisms that facilitate proactive avoidance of pathogens are widely accepted. How and when an individual acquires this proactive immune system, or if it is even ingrained into our genes, is yet to be determined.

As mentioned in *1.2.2.* disgust most likely stems from the primal function of distaste (Rozin et al., 2000), which can be found in animals and even in small babies (Berridge, 2000; Grill and Norgren, 1978; Steiner, 1973). However, further extension of disgust is only found later in life. The earliest evidence of disgust in children was found around the age of three years. Accordingly, 2.5-year-olds showed disgust in response to core disgust elicitors (i.e., dirt, spit, feces) and partially towards certain animals (i.e., cockroaches, maggots) but not towards sociomoral violations (i.e., theft, swearing). However, they only showed this at a rate of 50 %, which increased to 75 % by the age of 6.8 years (Stevenson et al., 2010b). Three-year-olds have been found to differentiate between good and bad smells in similar ways as adults (Schmidt and

Beauchamp, 1988). Further 3-year-olds were able to give contamination explanations for certain scenarios (i.e. Question: Why did the child get sick? Answer: Because the dog licked its candy.), but struggled with consistently making correct contamination predictions (i.e., Child X's, but not child Y's candy was licked by a dog, which child wild be sick tomorrow?) (Legare et al., 2009). Lastly, children between 2 and 4 years old seemed to struggle to recognize the typical disgust face, especially compared to other emotions, such as happy and angry, which already get recognized correctly most of the time at the age of 2 years. Only at 9 years old do children slowly start to recognize disgusted faces spontaneously (Widen and Russell, 2013). It has been found that young children's (Stevenson et al., 2010b) but also grown adults (Davey et al., 1993) reactions to animal and core disgust stimuli may be dependent on their parents' attitude towards the same stimuli, regardless of whether their parents were present during testing. If this is a heritable or a learned aspect, is yet to investigated. Yet, it seems like individuals develop food preferences, also regarding what they perceive as disgusting, from their family environment rather than from their genetic background (Rozin and Millman, 1987). In general, children (aged 7 to 10 years old) are able to learn from adults that certain animals are disgusting (Askew et al., 2014). However, data on disgust in children is surprisingly sparse, and to grasp a total understanding of disgust and contamination perception in children, further experimental research is needed.

Regardless of culture, some stimuli are rated as disgusting by most individuals. This includes bodily excretions and body parts, indicators of decay, and some specific living creatures (Curtis and Biran, 2001). However, certain cross-cultural variations may also seem to exist (Elwood and Olatunji, 2009), and especially so with other elicitors of disgust. In one study, only persons from western cultures (Germany, Spain, and the USA), but not from eastern cultures (China and Palestine), listed feces and rotten food as disgusting (Schweiger Gallo et al., 2024). It further seems that cultures differ in disgust sensitivity, so do Ghanaians show a significantly higher disgust sensitivity than US citizens. This is most prevalent in the subcategory of

contamination disgust (Skolnick and Dzokoto, 2013). The cultural differences may be due to environmental differences, with some cultures being more exposed to pathogens than others (Murray and Schaller, 2010).

Behavioral immune responses in individuals most likely develop in humans through a heritable basis and are extended by social learning (Fessler and Navarrete, 2003; Schaller et al., 2022) and/or through conditioning (Borg et al., 2016; Rozin, 1986). Pavlovian (classical) conditioning is a process defined by psychology where a neutral stimulus (eliciting no response) is linked to an unconditioned stimuli, that elicits a certain reaction, so that this reaction (conditioned response) is now also elicited by the now conditioned stimulus (previous neutral stimulus) (Rehman et al., 2025). It is well established that emotions can become conditioned responses. Pavlovian fear conditioning is most prevalent, often used in learning and memory studies on animal models. Fear conditioning is a fundamental learning process where a neutral stimulus becomes associated with an aversive event (such as an electroshock) through repeated pairings. Consequently, the previously neutral stimulus alone elicits a fear response, demonstrating the power of associative learning in shaping emotional reactions. (McCullough et al., 2016). Therefore, it seems reasonable to assume that humans can also show disgust as a conditioned response (a form of Pavlovian disgust conditioning). Experimental studies found evidence that disgust and associated avoidance can be learned via conditioning (Armstrong and Olatunji, 2017; Borg et al., 2016; Klucken et al., 2012). This may also be the basis for a proactive physiological immune response. As research on the proactive physiological immune response has just started, there are no studies on its evolutionary and/or learned aspects. To grasp a better understanding of the evolutionary background, studies in children, animals, and different cultures would be suitable. However, the upregulation of disease specific antibodies that are specialized even down to the protein, associated with the disease cue presented (*Chapter II;* Keller et al., 2023), suggest that the proactive physiological immune response is more similar

to the adaptive immune response that reacts to previously encountered pathogens, only in this case it reacts to previously encountered situations/ stimuli tied to the a certain class of pathogens. This would resemble a conditioning of an immune response. The brain encounters a neutral stimulus, like sneezing, that in the situation is associated with an unconditioned stimulus, such as a respiratory virus. The virus leads to an infection and therefore an immune response. After several repetitions, this may then lead to the sneeze becoming a conditioned stimulus and the immune response to become a conditioned (proactive) response that is elicited way before actual pathogen contact has been made. A mechanism, possibly akin to the conditioning of a proactive physiological immune response, is what researchers call immune conditioning. Here, the conditioned responses are immune and endocrine responses. In most studies, the neutral stimulus is something like a taste or a smell (Hadamitzky et al., 2020; Tekampe et al., 2017). This process has also been found to be associated with a heightened activation of the insula (Pacheco-López et al., 2005; Ramírez-Amaya et al., 1996; Ramírez-Amaya and Bermúdez-Rattoni, 1999). As previously discussed (see *1.2.4* and *3.1.4* as well as *Chapter 4*), the insula plays an important role in many immune and disgust-associated processes; our findings may be based on multiple processes connected with increased insula activation. It may be involved in learning (via conditioning (Klucken et al., 2012; Pacheco-López et al., 2005)) as well as storing and retrieving (Koren et al., 2021) the information that certain stimuli are associated with heightened infection risk and therefore modulate the immune response (Hess et al., 2011) by increasing, in our case, sIgA secretion in saliva (see Fig. 5 for overview). In order to test this hypothesis, further research will be necessary.

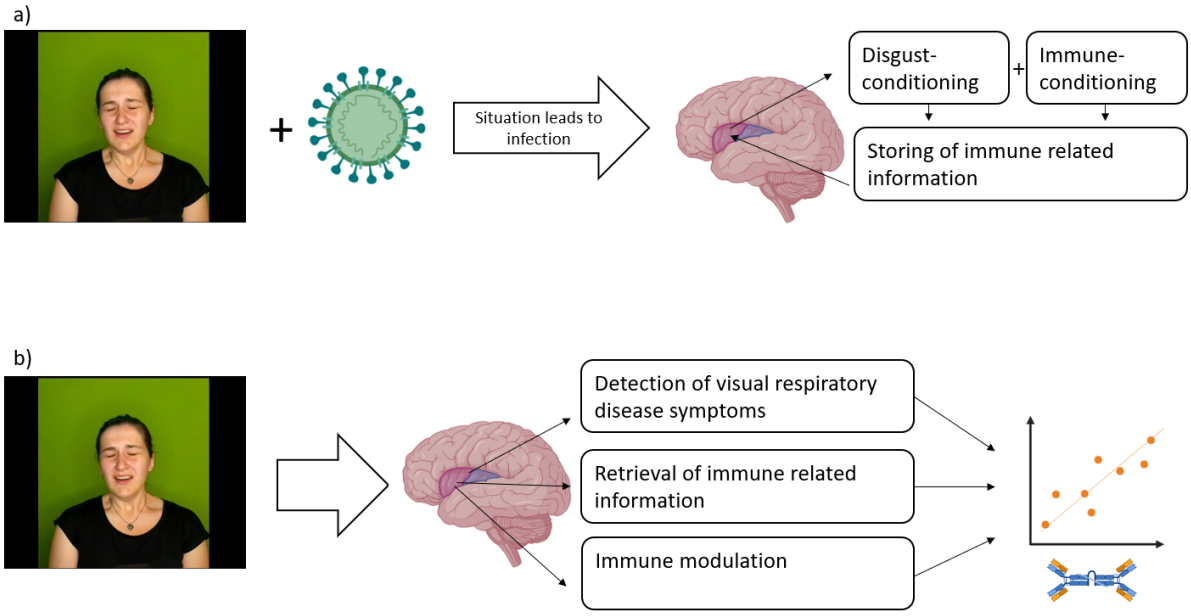

Figure 5: Overview of the possible process of conditioning a proactive physiological immune response. In step one (a), the neutral stimulus (person sneezing) is linked to the unconditioned stimulus (virus), which leads to the response of an infection; the information is most likely stored in the anterior insula. In step two (b), the now conditioned stimulus (person sneezing) leads to the recognition of the stimulus, the retrieval of stored information, and the proactive release of immune markers.

3.4 Conclusion

The findings of this thesis provide the first concrete and consistent evidence for a proactive physiological immune response in healthy humans. We could not only find a significant increase of total sIgA after visual disease cues (*Chapter I*), but also of pathogen-specific sIgA (*Chapter II*). Further, we found no evidence of an association between female sex hormones and this proactive physiological immune response (*Chapter III*). Lastly, we were the first to investigate the neural network that may underlie the proactive physiological immune response (*Chapter IV*).

Our results are now opening up a wide field of possible future research. Next to better understanding the mechanisms in healthy humans, by distinguishing which triggers stimulate which immune markers, understanding how long and effective this kind of physiological immune responses may be, and how the different kinds of immune responses (behavioral,

proactive, and reactive physiological) interact with each other. Our established test paradigm, through which disease cues reliably elicited a proactive mucosal and behavioral immune response, may also be useful to better understand altered patterns in patients with immune insufficiency in either behavioral or physiological immune responses. Lastly, how one may form these kinds of responses is still open, and the intra-individual variance is not understood yet.

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5. Figure & Table Index

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