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SCARE OR CARE?

HOW IMMUNOLOGICAL PROCESSES SHAPE PERCEPTION OF SICKNESS-RELEVANT STIMULI IN HUMANS

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Cover illustration: The picture shows a scene from the Caregiver Perception Task.
Both individuals have agreed to be part of the cover of this thesis.

Scare or care? How immunological processes shape perception of sickness-relevant stimuli in humans

Thesis for Doctoral Degree (Ph.D.)

By

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Till mamma.

Popular science summary of the thesis

What is the difference between the sick and the healthy you? Maybe there are things that you usually enjoy that feel less appealing when you are sick (hanging out with friends?), but also things that feel more appealing (binge-watching series on the sofa?). These changes in behavior during sickness are not random, it is a way for your brain to make sure that you behave in an optimal way while fighting the pathogen. The energy you usually spend while laughing with friends or running errands are now needed for your immune system. It is thus better to stay under the blanket. This thesis investigates how we see and interact with the world around us when sick. To explain our four studies, I need you think about the last time you had the flu.

Now imagine that you are lying on the sofa and starting the TV. A disturbing scene is shown, and you are feeling a bit upset, trying to tell yourself *"it is only a movie"* to calm down. In **Study I**, we let sick and healthy individuals watch unpleasant pictures, and asked them to *feel more* or *feel less* while watching. Interestingly, sick individuals reported that they were more successful in trying to feel less in response to the unpleasant pictures, compared to the healthy individuals. In other words, maybe your soothing sentence (*"it is only a movie")* is actually working better when you are lying feverish under a blanket.

You decide to leave the sofa and take a small walk outside to get some air. You are walking slowly. Feeling weak and tired. A couple walks past you, looking at you a bit suspiciously. You are wondering if they can see that you are sick. In **Study II**, we showed that humans are able to detect sick others based solely on the way they walk. So yes, it is actually possible that passing individuals may categorize you as sick.

Another person is now walking towards you. You wonder "*doesn't this person walk strangely?*". Maybe you feel like they walk like you, a bit slow, a bit rigid. Is this person also sick? Our results from **Study III** indicated that humans who are sick may more often perceive other healthy individuals as sick based on the way they walk. Possibly to be extra careful to not catch another pathogen when already sick and vulnerable. Maybe you should move a little further out on the pavement, just to be sure.

When you come home again, your partner is back from work. You move back under the blanket, feeling relieved, asking *"can you make me a cup of tea?"*. In **Study IV**, we showed that sick individuals are willing to receive care from others who may be especially prone to help, even if they are not familiar with the possible care provider, *and* even if the care provider is not a healthcare professional. In other words, if your partner is not fast enough with that cup of tea, you may find yourself considering asking someone else for help.

Altogether, these studies show how things can appear differently for the sick individual, and how the sick individual also can be perceived differently by others. Clearly, sickness does not make everything grey. Some things, like a supportive partner, can look even brighter.

Abstract

Humans and other animals have developed several defense systems to handle living in a pathogen-rich world. These defense systems include immune responses, as well as behavioral responses aimed at supporting immune functions during the fight against the infection, called *sickness behavior*. Since sickness behavior is believed to be adaptive, it is possible that sickness shifts perception of the world depending on the priorities and needs of the sick individual. For instance, sick humans in general avoid social interactions to save energy, but can also approach specific people that can provide care and support. However, these ambivalent aspects of sickness behavior are understudied. Thus, the aim of this thesis was to investigate perception of sickness-relevant stimuli, and how such perception is modulated during immune activation. In particular, we assessed how immune activation affected cognitive reappraisal of emotions to unpleasant stimuli (**Study I**), if naïve observers can detect sick others and if this ability is affected by immune activation (**Studies II-III**), and if immune activation affects perception of unfamiliar caregivers (**Study IV**).

In four studies, we used the model of experimental endotoxemia, consisting in intravenously injecting a low dose of the bacterial endotoxin lipopolysaccharide (LPS) into healthy volunteers. The recognition of LPS by immune cells triggers inflammatory responses, and causes a transient state of sickness for a few hours, allowing studying sickness behavior in an experimental setting. In **Study I**, participants received an LPS or a saline (placebo) injection, and completed a task in which they were asked to down-regulate or up-regulate their emotions in response to general negative and disgust stimuli. We showed that sick participants reported a greater success in *down-regulating* their emotions to general negative and disgust stimuli, compared to healthy participants. In **Studies II-III**, we used sickness detection tasks, in which naïve observers rated the health status of stimuli consisting of photos of faces and video recordings from a walking task obtained from the participants in Study I. In **Study II,** naïve observers could detect sick others solely from the way they walked. In **Study III**, participants performed a sickness detection task, once when sick (LPS injection) and once when healthy (no injection). We showed that, when sick themselves, individuals categorized more healthy walkers as sick, and were thus less good at discriminating between sick and healthy walkers, compared to when healthy. In **Study IV**, we developed the Caregiver Perception Task (CgPT), which participants completed when sick (LPS injection) and when healthy (saline injection). The findings revealed that sick participants were more willing to receive care from unfamiliar care providers, compared to when healthy.

This thesis adds to the current knowledge on *social sickness behavior*. Altogether, these findings highlight that sickness is not all about perceiving the world as more negative. Yes, sick individuals may categorize others more easily as threats, but sickness can also possibly increase the ability to *feel less* negative emotions, together with making some items and individuals in the environment more appealing (e.g., caregivers). Future studies need to investigate how such changes in perception of sickness-relevant stimuli translate into behavior.

List of scientific papers

I. **Hansson LS**, Axelsson J, Petrovic P, Paues Göranson S, Olsson MJ, Lekander M/Lasselin J. (2021). Regulation of emotions during experimental endotoxemia: A pilot study. *Brain, Behavior, and Immunity*, 93, 420-424. doi:10.1016/j.bbi.2021.01.013.

II. **Hansson LS**/Lasselin J, Tognetti A, Axelsson J, Olsson MJ, Sundelin T/Lekander M. (2023). The walking sick: Perception of experimental sickness from biological motion. *Brain, Behavior, and Immunity*, 113, 319-327. doi:10.1016/j.bbi.2023.07.020.

III. **Hansson LS**, Tognetti A, Tavakoli E , Stache J, Kakeeto M, Melin J, Bredin S, Skarp R, Lensmar C, Demand R, Olsson MJ, Wilhelms DB, Toll John R, Jensen K, Lekander M/Lasselin J. Identifying sick people while sick yourself: a study of identification of facial cues and walking patterns of sick individuals during experimental endotoxemia. *Draft manuscript.*

IV. **Hansson LS**, Tognetti A, Sigurjónsson P, Brück E, Wåhlén K, Jensen K, Olsson MJ, Toll John R, Wilhelms DB, Lekander M/Lasselin J. Perception of unfamiliar caregivers during sickness – using the new Caregiver Perception Task (CgPT) during experimental endotoxemia. *Accepted for publication in Brain, Behavior, and Immunity.*

Contents

List of abbreviations

ACC	Anterior cingulate cortex
BBB	Blood-brain barrier
BMI	Body mass index
BIS	Behavioral immune system
bw	Body weight
CgPT	Caregiver Perception Task
COVID-19	Coronavirus disease 2019
CNS	Central nervous system
GDPR	General data protection regulation
IFN	Interferon
IL	Interleukin
LPS	Lipopolysaccharide
OSF	Open science framework
TLR	Toll-like receptor
TNF	Tumor necrosis factor
PAMP	Pathogen-associated molecular pattern
PLD	Point-light display
PFC	Prefrontal cortex
PRR	Pattern recognition receptor
SicknessQ	Sickness Questionnaire
VAS	Visual analogue scale

Preface

I started my PhD in April 2020, a time when the COVID-19 pandemic was hitting the world. I remember that I thought a lot about how pandemics differ from other catastrophes. In my mind I saw images from horrible events, like natural disasters and terror attacks, but these pictures also consisted of people standing in groups, hugging, or holding each other's hands. This was different. All over the world, people were alone in their homes. Like scattered human islands of fear. Always looking at each other from a distance, through a screen, or a plastic protection. Instead of being scared together, we were scared of each other. Our covered faces examining each other for signs of sickness. Scared, yes, but at the same time longing for each other, caring for each other. Will someone bring me food if I get sick? What if I infect someone I love?

When I tested positive for COVID-19 a year later, I thought about how my feelings and behaviors were shifted. Instead of running around, I was lying on the sofa under a blanket. Instead of eating a mixed diet, I only wanted to eat chocolate pudding and ice cream. My body and mind felt slow. The things I usually enjoy, like reading or dancing, no longer felt appealing. Why do anything when you can sleep? I did not answer to any texts from my friends. But I missed my mother.

When I felt a little bit better, I took a picture of myself outside in the sun. I sent the picture to my principal supervisor with the text *Sick or Healthy?* I look pale in the picture. My eyes watery and red. A bit sweaty. Maybe a bit sad? Sad and tired. My sickness was not only within my body, it was showcasing itself to the world. In the way I looked, but also in the way I behaved. The slow movements, the distancing. The world looked different to me, but I also looked different to the world.

April 2020 was the worst of times, but maybe the best of times to start a PhD that touches upon many of the personal experiences described above. The COVID-19 pandemic featured the relevance of studying how healthy people appraise sick people, and how the sick perceives the world. In some ways, the locked down world opened up the future for our world, the world of psychoneuroimmunology.

1 Background

If I ask you how humans and other living species cope with a world full of pathogens, the first thing that will come to mind is probably the immune system. Depending on your knowledge level, the concept of the immune system may range from an idea of a diffuse complex biological system, to detailed illustrations of various cell types and signaling pathways. Indeed, humans and other animals have developed an impressive system of cells and physiological functions that will recognize and attack intruding pathogens. What may be less obvious, is the complementary *behavioral* strategies that have evolved to protect the body from pathogen threats. First, animals have developed *proactive behavioral defenses*, allowing them to detect and respond to pathogen cues (Schaller, 2011). Yet, even with this system, pathogens will constantly enter the body via different routes. The influx and proliferation of pathogens will then activate immune cells, but also trigger *reactive behavioral changes*, aimed at minimizing the harm from the infection (Hart, 1988). These specific changes are known as *sickness behavior* (Dantzer, 2001). My own experience with COVID-19, as described in the preface, captures all these defense systems. I tried to avoid catching the virus (proactive defenses), but in the end I was still infected and my immune system was triggered, causing distinct behavioral changes (sickness behavior). While being sick, my appearance also changed, possibly making others avoid me. And the circle is complete.

1.1 Proactive behavioral defenses

Have you thought about the way you scrunch your nose when smelling rotten food? Or why you might change seat in the bus when the passenger next to you is coughing? These are examples of proactive behavioral defenses against diseases, which consist of mechanisms aimed at distancing us from pathogen threats in our surroundings (Schaller, 2011). This disease avoidance system has sometimes been termed *the behavioral immune system (BIS)*, and comprises the ability to detect possible infectious items and individuals, alongside with eliciting emotional and behavioral responses upon detection, such as feelings of disgust and subsequent avoidance behaviors (Schaller & Park, 2011).

1.1.1 Detection and avoidance of sick individuals in non-human animals

Animals express prophylactic behaviors, such as grooming and fly-repelling behaviors, aimed at removing pathogens (Hart, 2011). Yet, this may not be enough for animals that live in social groups. Sociality provides many benefits, such as protection from predators, but it has the downside of adding contagious individuals to the list of contamination threats (Townsend et al., 2020). Strategies such as avoiding individuals detected as sick are thus widespread across social species (Stockmaier et al., 2021). For instance, healthy rodents distance themselves from sick conspecific (Hamasato et al., 2017; Renault et al., 2008), and prefer interacting with healthy conspecifics compared to sick (Rieger et al., 2022). Avoidance may also be expressed as decreased physical contact. For example, a study in wild mandrills showed that infection status interfered with grooming behavior, as peers decreased their

grooming of contagious others, at least when non-kin (Poirotte & Charpentier, 2020). These findings illustrate how animals may adapt their social behaviors to avoid contagious others.

The ability to detect and avoid contagious conspecifics has also been observed in non-mammals, such as bullfrog tadpoles (Kiesecker et al., 1999), social lobsters (Behringer et al., 2006), and birds (Love et al., 2021). How is it possible for this vast number of species to distinguish sick individuals from others? In many cases, the answer lies in the nose. In fact, odors are believed to serve as the most important sickness cue[1] in non-human animals (Butler & Behringer, 2021). Yet, one study in fish and one study in birds showed avoidance of sick individuals even if all chemical cues were blocked with a glass wall, illustrating that animals can detect sick individuals through several sensory modalities (Love et al., 2021; Stephenson et al., 2018). From birds in the trees, to fish in the sea, and in many animals in-between: the ability to detect and avoid sick individuals is clearly everywhere in the animal kingdom, and humans are no exception.

1.1.2 Detection and avoidance of sick individuals in humans

Do you believe that you have the ability to detect sick individuals in your surroundings? Based on responses from a recent survey, the answer to this question seems to be *yes* for many people, at least with respect to visual and auditory cues of sickness (Ackerman et al., 2020). When put to the test, naïve observers were indeed able to accurately identify individuals who were made sick experimentally (see part 1.3.1) (Arshamian et al., 2021; Axelsson et al., 2018; Tognetti, et al., 2023a), or who were sick in natural settings (Leung et al., 2023), from photos of faces. On the contrary, no study has yet proven that humans are able to detect sick individuals from the way they sound. When listening to sneezes and coughs from individuals with an infectious disease and from individuals sneezing and coughing for other reasons (e.g., allergy), the participants did not discriminate between contagious and non-contagious sound sources. Instead, participants rated disgust-evoking sounds as more contagious, independent of the infectiousness of the source (Michalak et al., 2020).

Even if odors clearly are important sickness cues for other animals (Butler & Behringer, 2021), humans seem to underestimate their ability to *smell sickness*. In the same survey as mentioned above, people did not judge smell as an effective sense for sickness detection (Ackerman et al., 2020). Yet, several studies show that odor cues may provide important information about humans' health status. For example, participants rated odors from individuals made sick experimentally as more intense, less pleasant, and less healthy, compared to odors from the same individuals when they were healthy (Gordon et al., 2023; Olsson et al., 2014). Similarly, raters could discriminate between odor samples collected from individuals with naturally occurring respiratory infections and samples from the same

[1] Of note, the term "cues" is used here to separate the transferred information from "signals" which have developed to alter behavior in the perceptual receiver (Maynard-Smith et al., 2003).

individuals when healthy, although the effect was small (Tognetti, et al., 2023b; but see Sarolidou, et al., 2020a). These findings may be understood in the light of studies showing that volatile organic compounds, constituting body odors, are affected by different health conditions (Shirasu & Touhara, 2011).

Altogether, humans appear able to detect sick individuals, but will such detection translate into avoidance? In humans, behavioral avoidance of sick individuals has not been extensively studied. Instead, subjective ratings, such as liking, have often been used as a proxy for such behavior. Unsurprisingly, if you like someone, you may be more prone to approach this person (Cialdini & Goldstein, 2004). The observed decreased likability of sick individuals, compared to healthy individuals, based on photos of faces (Leschak et al., 2022; Regenbogen et al., 2017; Sarolidou et al., 2020b) may thus indicate tendencies of increased avoidance of sick individuals. Some studies have also used subjective ratings more directly related to behavioral avoidance. For instance, participants rated sick individuals as less socially desirable (Regenbogen et al., 2017), and as more likely to be avoided (Leung et al., 2023), compared to healthy individuals. In a recent study, adults and children saw pictures of "twin pairs", which in reality consisted of the same individuals when sick and when healthy. When asked to select which twin they wanted to sit next to during an imaginary dinner, both adults and children preferred healthy individuals, compared to the same individuals when sick (Leung et al., 2024). Most of the studies described above used sick faces with subtle sickness cues, such as paleness and droopy mouth corners (Axelsson et al., 2018). Yet, other studies have used more prominent cues of sickness. For instance, findings from one such study showed that participants were less comfortable with being close to individuals with rashes, compared to individuals without such sickness cues (Bressan, 2021; van Leeuwen & Petersen, 2018). Altogether, these findings emphasize that humans may avoid sick others. In addition to subjective ratings, the feeling of disgust can also be used as a proxy for avoidance behavior.

Can you make a face of disgust? Wrinkle your nose, raise your upper lips, and narrow your eyes. Great, now you can stop. This facial expression of disgust has been argued to look similar across many regions around the globe (Ekman & Friesen, 1971; Rozin et al., 1994), and triggered by similar objects (Curtis & Biran, 2001), underlining disgust as a central emotion. It is not surprising that disgust is a core aspect of proactive behavioral immune defenses, given that objects which elicit disgust, such as spoiled food and bodily fluids, are pathogen threats (Curtis & Biran, 2001). Disgust may even have developed with the function to allow for avoidance of infectious and poisonous threats (Oaten et al., 2009). Indeed, there seems to be a direct link between disgust and avoidance. For instance, participants looked less at videos displaying vomit compared to other types of videos, and such *visual avoidance* is more prominent in more disgust-sensitive individuals (Armstrong et al., 2014). Several studies have also investigated how far individuals are willing to go while interacting with disgust-evoking items. In one such study, participants received a bag with possibly contagious items, and were asked to get closer to the items by following several steps. First by touching the bag, then by removing the items from the bag, then by putting their mouth on

their hand which had touched the items, and finally touching the items with their mouth. (Are you making that face of disgust again?) Results from this study revealed that individuals with higher disgust sensitivity stopped earlier, and thus were more avoidant of the items (Fan & Olatunji, 2013). Interestingly, individuals with high disgust sensitivity appear to be subjected to less infections, at least when living in pathogen-rich environments, suggesting that disgust may protect against infectious diseases (Cepon-Robins et al., 2021).

In addition to items, contagious others may also provoke disgust, and thus avoidance. When presented with pictures of faces with various degrees of visual sickness cues, participants rated faces with more distinct sickness cues (e.g., rashes) as more disgusting (Hedman et al., 2016). This was actually not as clear in another study in which participants rated subtle visual and olfactory cues from sick and healthy individuals, while their facial muscles were recorded to capture expressions of disgust. Results showed that the facial disgust expression did not relate to the dislike of sick individuals, indicating that aversion of subtle sickness cues may not be strongly driven by disgust (Sarolidou et al., 2020b).

Altogether, these findings indicate that humans and other animals are able to detect, and likely avoid, disease threats. Pathogens which escape such proactive behavioral defenses, and succeed in entering the body, will encounter the next line of defense – the immune system.

1.2 The immune system

Immunity exists in all life forms. The strategies may be different, but they all have developed from the need to protect a "self" constantly surrounded by pathogens (Danilova, 2006). Similarly to many animals, humans have both an innate and an adaptive branch of defense (Abbas et al., 2014). The innate immune response is fast but standardized across many pathogen threats, whereas adaptive immunity is slow but highly specific to each pathogen threat (Clark & Kupper, 2005). When a new pathogen enters the body, the cells of the innate immune system will recognize it and attack it immediately. Thus keeping guard, while the adaptive system prepares cells that are aimed at specifically combating the new threat. The two branches thus complement each other, providing us with a system that can act immediately, but also learn and adapt as we go through our pathogen-rich life.

1.2.1 Innate and adaptive immune responses

A pathogen that has overcome avoidance and physicals barriers (e.g., the skin, the respiratory mucosa, and the cells lining the gastrointestinal track) will be recognized by several types of innate immune cells (Akira et al., 2006). Many of these cells are *phagocytes*, meaning that they fight intruding pathogens by "swallowing" (phagocyting) them. Two such cell types are neutrophils and monocytes, which are moving in the bloodstream, and scavenging for intruders. Neutrophils and monocytes can also be recruited into the tissue, upon which monocytes differentiate into macrophages. Macrophages, together with dendritic cells, are patrolling the tissues of the body organs, engulfing pathogens, and initiating immune signaling pathways. But how can these cells recognize that the pathogens moving inside us are not part of us?

Pathogens have specific characteristics in their constructions, which flags them as belonging to something other than the self. Such characteristics are known as pathogen-associated molecular patterns (PAMPs) and are recognized by specific receptors on innate immune cells (pattern-recognition receptors, PRRs). One PAMP that is particularly important for the current thesis is a bacterial endotoxin called lipopolysaccharide (LPS), which is a component of the outer membrane of gram-negative bacteria (Beutler, 2009). In the nineties, a vivid hunt for the LPS-sensing receptor was initiated by Bruce Beutler and his team. Five years of gene hunting later, they found what they were looking for, the toll-like receptor (TLR)-4 (Poltorak et al., 1998). This work largely built upon findings from Jules Hoffmann and his team, which discovered the importance of the Toll receptors for immune functions in fruit flies (Lemaitre et al., 1996). These discoveries later turned both Beutler and Hoffmann into Nobel prize laureates, "*for their discoveries concerning the activation of innate immunity*" (Ravindran, 2013). Since then, several other TLRs have been discovered and functionally described. Indeed, the TLR family is today recognized as an important class of PRRs, which recognize bacterial lipids and proteins, as well as viral components (O'Neill et al., 2013).

The binding of PAMPs to PRRs results in the release of small messenger proteins, known as *cytokines*, that can initiate systemic inflammation (see part 1.2.2). Additionally, the binding

and engulfing of pathogens also serve as a link between innate and adaptive immunity. Although adaptive immunity is not of high relevance for the present thesis, its importance for immune functions demands for a brief introduction. Innate immune cells do not only phagocyte ("swallow") pathogens, they also bring very small and specific parts of these engulfed pathogens to their cell surfaces (Abbas et al., 2014). These *antigens* are recognized by antigen receptors on the adaptive immune cells: B-lymphocytes and T-lymphocytes. You most likely have heard about the antigen receptors on the surface of B-lymphocytes, known as *antibodies*. These receptors are released into bodily fluids to neutralize pathogens or to mark them for destruction. T-lymphocytes have bound receptors that, upon binding with an antigen, trigger several immune functions, such as destruction of infected cells and release of cytokines (Abbas et al., 2014). The activation of the adaptive immune branch will induce proliferation of cells with receptors specialized for the specific antigen. When the pathogen is removed, some of the specialized cells and antibodies will remain, ready to act upon reinfection (Abbas et al., 2014). The adaptive system thus provides an *immunological memory*, which remembers our old battles, and prepares us for the ones to come (Abbas et al., 2014). Yes, it is impressive indeed, but let us now go back to the innate immune system.

1.2.2 Inflammation

Already in ancient times, humans were trying to describe inflammation based on what they could see and feel. Think about the last time you had a wound. What did the skin around the wound look like? Red and swollen. How did it feel when you touched the skin? Warm and painful. How was the injured body part affected? It was harder to move it. You are thinking like the ancient Romans, who described the signs of inflammation as *rubor* (redness), *tumor* (swelling), *calor* (heat), *dolor* (pain), and *functio laesa* (loss of function). Today, we know that these symptoms are caused by the increased blood flow to the area, the infiltration of immune cells, and the effects on surrounding tissue (Punchard et al., 2004). Innate immune cells that are recruited to the affected area will release inflammatory mediators, such as cytokines. If these cytokines reach the circulation, the local inflammation will transform into systemic inflammation (Medzhitov, 2008).

Cytokines are small proteins responsible for various immune functions. These signaling molecules can act both close to their secretion site (i.e., autocrine or paracrine signaling) but also travel in the bloodstream to act on distant sites (endocrine signaling) (Abbas et al., 2014). During acute inflammation, innate immune cells release a specific set of cytokines, namely tumor necrosis factors (TNF), interferons (IFN), and several interleukins (IL), such as IL-1, IL-6, IL-8, and IL-10. Most of these cytokines are pro-inflammatory, meaning that they are triggering and amplifying the inflammatory response. Other cytokines, such as IL-10, are anti-inflammatory, inhibiting the production of pro-inflammatory cytokines and regulating the inflammatory response (Abbas et al., 2014).

Although the inflammatory responses are classically described as local and systematic responses, as mentioned in the introduction of 1.1, the immune responses are accompanied by

a behavioral response called *sickness behavior.* Yes, immune responses to pathogens occur peripherally, but also in the brain.

1.2.3 Immune-to-brain and brain-to-immune signaling

As we move around in the world, our immune system is within us, each moment representing a unique snapshot of our immune status. These snapshots are not concealed from our brain. Instead, the immune system and the brain are constantly interacting in a bidirectional manner. When a pathogen enters the body, the brain will receive this information, and will communicate back to the immune system to modulate the immunological response (Dantzer et al., 2008). But how can the brain receive information about the influx of pathogens? The answer lies in the small messenger proteins described above, the cytokines.

Peripheral cytokines can signal to the brain via neural, hormonal, and cellular pathways (see **Figure 1**). Circulating cytokines can bind to receptors expressed on afferent nerves (e.g. the vagus nerve), triggering a peripheral signal which travels along the nerves to the brain (the neural pathway). The cytokines may also directly enter the brain via different routes (the hormonal pathway), and immune cells can be recruited to the brain (the cellular pathway) (D'Mello & Swain, 2017). Thus, peripheral cytokines can be "sensed" by the central nervous system, and in turn modulate brain activity.

Signaling via the vagus nerve is one important example of immune-to-brain communication via the neural pathway (see **Figure 1A**). The vagus nerve branches all across the body, covering most of the visceral organs, including the heart, the lungs, and the gastrointestinal track. Signals from these vast bodily locations cumulate in the brainstem, and are then projected across the brain (Berthoud & Neuhuber, 2000). The role of the vagus nerve in the neural pathway has been investigated with experiments using vagotomy in rodents, in which parts of the vagus nerve was surgically removed (Konsman et al., 2002). Hence, researchers determined that inflammation-induced behavioral changes (i.e. sickness behavior, see part 1.3) were blocked when intraperitoneally (via the abdomen) injecting LPS or the cytokine IL-1β into vagotomized rats (Bluthé et al., 1994; Konsman et al., 2000). Since then, several studies have used models with vagotomy together with injections of LPS or with the cytokine IL-1β, to demonstrate the importance of the vagus nerve in immune signaling from the abdomen (Dantzer et al., 2000). Yet, when injecting IL-1β intravenously, instead of intraperitoneally, to vagotomized rats, the inflammation-induced behavioral changes remained, thus suggesting the involvement of other immune-to-brain routes (Bluthé et al., 1996).

The brain is a unique organ in its importance and complexity. Thus, it is not surprising that it is uniquely protected. The brain and the spinal cord (i.e., the central nervous system; CNS) are guarded by layers of tightly connected endothelial cells, the blood-brain barrier (BBB). This barrier does not allow for vast influx of immune cells or cytokines from the blood to the brain. Still, circulating cytokines may slip in via certain routes. For instance, the BBB has some parts which are less tight (i.e., the circumventricular organs), and the cytokines are free

to slowly diffuse through these leaky parts (D'Mello & Swain, 2017). Additionally, there are active transporters allowing the passing of cytokines across the BBB (D'Mello & Swain, 2017). This direct influx of peripheral cytokines into the brain parenchyma, is known as the hormonal pathway (Dantzer et al., 2008) (see **Figure 1B**).

On the CNS side of the BBB, the brain's own immune cells are patrolling. These cells are a modified version of macrophages, known as microglia. Microglia protect the CNS by scavenging for pathogens and injuries (Li & Barres, 2018). When the peripheral cytokine signal is communicated to the brain via the neural and hormonal pathways, the microglia are activated (Hoogland et al., 2015), triggering the release of cytokines and other immune mediators (D'Mello & Swain, 2017). The activation of microglia also results in the release of chemoattractant cytokines, calling for monocytes in the blood to diffuse across the BBB. These migrating monocytes will release cytokines directly into the brain, and thus amplify the cytokine signaling (D'Mello & Swain, 2017). This cellular pathway thus allows for some peripheral immune cells to act from within the brain (D'Mello & Swain, 2017) (see **Figure 1C**).

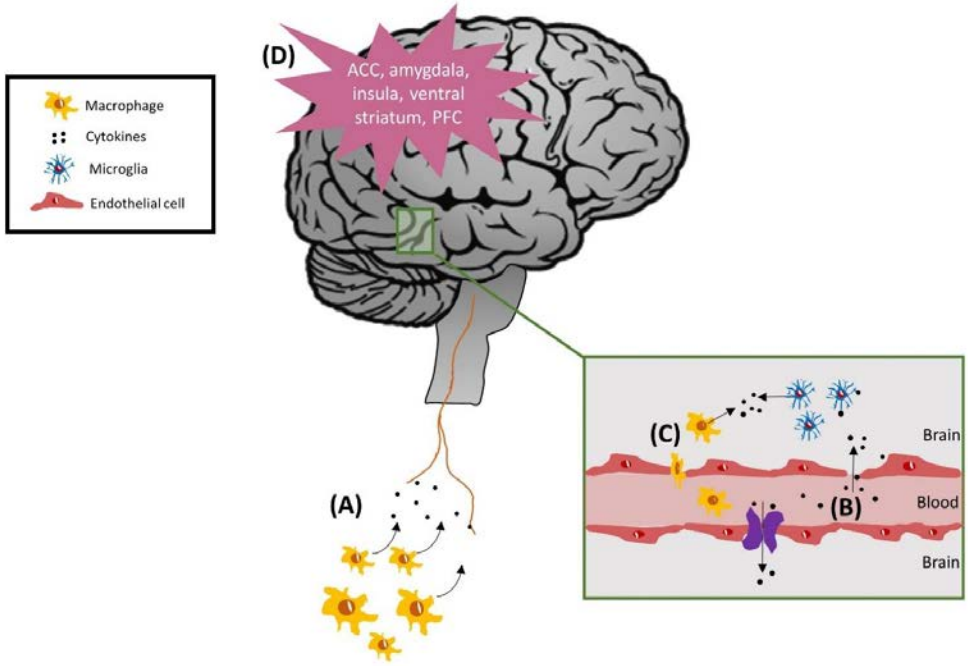

Figure 1. Immune-to-brain communication. Peripheral cytokines signal to the brain via three pathways: (A) stimulation of afferent neurons (neural pathway), (B) direct entering of the brain via active transporters and diffusion through leaky parts of the blood-brain barrier (hormonal pathway), and (C) recruitment of monocytes to the brain (cellular pathway). These pathways activate microglia, the brain's own immune cells, which upon activation release cytokines directly into the brain. The peripheral inflammatory signaling affects several brain regions important for mood and behavior (D). Abbreviations: ACC: anterior cingulate cortex. PFC: prefrontal cortex. Credit: Lina Hansson.

As described above, the cytokine signal from the periphery reaches the brain, and is also amplified at the periphery via several pro-inflammatory pathways. But more is not always better when it comes to cytokines. In fact, a major release of pro-inflammatory cytokines, a *cytokine storm*, is a severe and possibly mortal condition (Cron et al., 2021). There are thus several regulatory systems in which the brain talks back to the immune system, i.e., brain-to-immune signaling. One such system is the *inflammatory reflex*, the cholinergic anti-inflammatory pathway described by Kevin Tracey and his team (Tracey, 2002). Remember the vagus nerve? This nerve does not only transmit information to the brain, it also consists of an efferent part, signaling from the brain to the periphery. This efferent part of the vagus nerve constitutes a main component of the so-called parasympathetic nervous system known to control various bodily functions, such as digestion. In the cholinergic anti-inflammatory pathway, efferent fibers of the vagus nerve signal to splenic nerves. This signaling recruits T-lymphocytes, which produce the neurotransmitter acetylcholine, which in turn downregulate the production of pro-inflammatory cytokines from macrophages (Rosas-Ballina et al., 2011). Immune functions are also regulated via the activation of the hypothalamic-pituitary-adrenal axis, triggering the release of cortisol, and via the activation of the sympathetic nervous system with the release of adrenaline and noradrenaline (Tracey, 2002). Altogether, these systems help to fine-tune the inflammatory response.

The incoming inflammatory signals from the periphery have substantial effects on brain processes. For instance, such signals are known to affect the release of several neurotransmitters important for mood and behavior, including serotonin, glutamate, and dopamine (D'Mello & Swain, 2017). Findings from both animal models and human studies have pinpointed a large set of brain regions which are affected by peripheral inflammation, including hypothalamus, amygdala, anterior cingulate cortex, thalamus, and the prefrontal cortex (Kraynak et al., 2018) (see **Figure 1D**). These regions regulate motivational drive, movement, attention, and sociality, all key aspects of sickness behavior (Harrison, 2017).

1.3 Sickness behavior

When I started to learn about sickness behavior, the first paper I read was a groundbreaking paper from Benjamin Hart (Hart, 1988). This paper has an illustration of a dog, lying alone in a curled-up position, next to its untouched bowl of food (see **Figure 2**). The face has a painful expression, and the body is shaking. It is fascinating how this small number of lines can convey many of the core aspects of what it means to *feel* and *act* sick. Indeed, if we go back to my own experience with COVID-19 described in the preface, the similarity with the dog's behavior is striking. The sickness also changed the way I moved, ate, and socialized.
I had transformed into the Hart-dog.

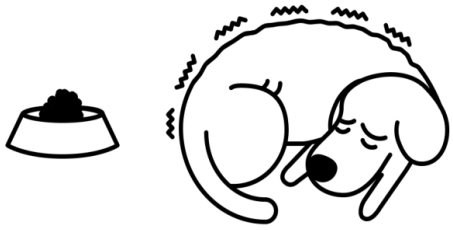

Figure 2. A sick dog. Illustration of a sick dog inspired by a figure in Benjamin Hart's *Biological basis of the behavior of sick animals* (Hart, 1988). Credit: Johan Skär Holm.

Becoming sick is an inevitable part of life. Thus, it is important to study how the state of sickness affects the way the individual perceives the world. Furthermore, comprehending behavioral changes in sociality is important to understand how contagious diseases are spread between individuals. To study sickness behavior in detail and with experimental scrutiny, researchers, including me, initiate such behavioral changes in animals and humans in an experimental way.

1.3.1 Studying sickness behavior with the model of experimental endotoxemia

The first time I observed an injection of LPS into a human was in the autumn of 2021. After reading numerous papers using the model, I was nervous and eager to see it with my own eyes. During the first hour following the injection, nothing happened. I was sure that the participant had received a placebo, since they were energetic and joking with the medical doctor. Yet, after one hour, there was a sudden switch. The participant, who had started to feel shaky and tired, now wanted to lie down in bed. They no longer talked to the medical doctor, nor looked at their phone. The effect of the LPS injection had kicked in, the sickness behavior had started.

The model of experimental endotoxemia (or "the LPS model") was first used in animal models in the middle of the 20th century. Since then, it has been the most used model to experimentally induce and study sickness behavior (Lasselin et al., 2020a). The reason why I wanted to introduce you to the PAMP of the gram-negative bacteria earlier was because this

model consists of injecting intravenously a small dose of this bacterial endotoxin LPS into healthy volunteers. As described above, LPS is part of the outer membrane of gram-negative bacteria and binds to TLR-4 receptors on innate immune cells. The binding of LPS triggers the release of cytokines by immune cells, which signal to the brain and affect brain regions important for mood and behavior, in similar ways as during an infection although in a much shorter timeframe (see **Figure 3**) (Lasselin, et al., 2020b). Thus, the path from an intruding pathogen to sickness behavior is initiated, even if no actual pathogen is present.

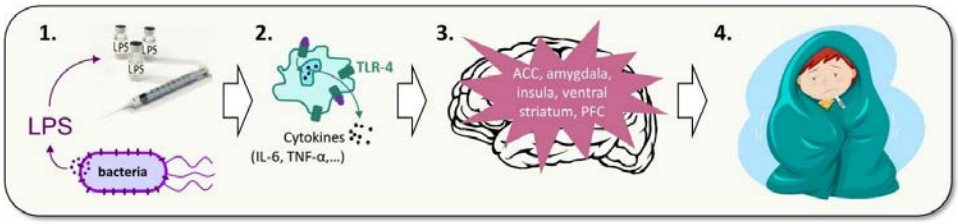

Figure 3. The model of experimental endotoxemia. In the model of experimental endotoxemia, lipopolysaccharide (LPS), a bacterial fragment of gram-negative bacteria, is injected intravenously into healthy volunteers (1). Injected LPS binds to TLR-4 on immune cells (e.g. macrophages), triggering the release of peripheral cytokines (2). The peripheral signal affects several brain regions important for mood and behavior (3), and gives rise to sickness behavior (4). Abbreviations: LPS: Lipopolysaccharide, IL: interleukin, TNF: tumor necrosis factor, TLR: toll-like receptor, ACC: anterior cingulate cortex. PFC: prefrontal cortex. Credits: Julie Lasselin. Illustration of sick person: brgfx/Freepik.

In humans, the injection of LPS induce core aspects of sickness behavior, such as negative mood, fatigue, decreased appetite and social withdrawal (see part 1.3.2) (Lasselin, et al., 2020b). The symptoms are transient and vanish after approximately five hours, enabling an accelerated version of infection-like sickness that is viable in experimental settings (Lasselin, et al., 2020b). Although the model of experimental endotoxemia is the focus of the present thesis, it should be mentioned that several vaccine models have been used to study inflammatory-induced behavioral changes in humans. Vaccine models (e.g., typhoid vaccination or influenza vaccination) initiate a low-grade inflammatory response, which is followed by behavioral changes (Harrison et al., 2009; Jolink et al., 2022). Such changes are very mild compared to the model of experimental endotoxemia, and the vaccine models are thus not as suitable for studying sickness behavior during acute sickness.

1.3.2 Sickness behavior as a motivational state

Sickness behavior is the inflammation-induced changes in mood and behavior observed in sick animals (Dantzer & Kelley, 2007) – but what do these changes comprise more specifically? If we go back to the illustration of the dog (see **Figure 2**), we can note three things: the dog is lying down in a curled-up position, the food next to the dog is untouched, and the dog is alone. This leads us to three distinct parts of sickness behavior – *lethargy*, *anorexia*, and *social withdrawal* (Dantzer, 2023; Hart, 1988). First, lethargy is a slowed down state of being that can be seen across species in sick animals (Lopes et al., 2021), and can be translated to the subjective feelings of fatigue and sleepiness in sick humans (Lasselin, Karshikoff, et al., 2020). Secondly, anorexia relates to the decreased food intake observed in

sick animals and the reported loss of appetite in humans (Lasselin, et al., 2020a). Thirdly, social withdrawal relates to the decreased interest in social activities observed in sick animals (Avitsur & Yirmiya, 1999; Fishkin & Winslow, 1997), and the feelings of social disconnection and the willingness to be alone in humans (Eisenberger et al., 2010; Hannestad et al., 2011). These behavioral changes in the sick animal is often disregarded as an insignificant add-on to the state of sickness. Yet, the overlap in inflammation-induced behavioral changes across species might give a hint about a possible adaptiveness of such behavior (Lopes et al., 2021).

The dog in the illustration is also feverish, shivering from the increase in body temperature. Fever is an important physiological response, which favors the activity of innate and adaptive immune cells, and several studies have shown that the increase in body temperature is important for survival (Harden et al., 2015). But this increase in body temperature is also energetically costly (Baracos et al., 1987). Thus, sickness behavior has been argued to consist of an adaptive set of changes aimed at helping the animal to preserve body energy in order to fight the pathogen and to promote recovery (Dantzer, 2001; Hart, 1988). Indeed, the decreased investment in locomotion, foraging, and social interactions would arguably spare energy which could be used by immune cells to fight off the pathogen (Dantzer, 2001). Additionally, a reduction in food intake may "starve" the pathogen and thus be beneficial (Hite et al., 2020), at least for bacterial infections (Dantzer, 2023).

The manifestation of sickness behavior is nevertheless dependent on the context. In one early study, researchers observed how the investment in nest building by mice dams was affected by inflammation and temperature. Results showed that mice dams injected with LPS and housed at a comfortable room temperature spent less time building nests for their pups, compared to healthy dams. Yet, when the temperature was decreased to 6°C, there was no difference in investment in nest building between sick and healthy dams (Aubert et al., 1997). In addition to temperature, the social environment may affect how sickness behavior is manifested. Zebra finches (the birds, not the fish) in isolation increased their resting time after an LPS injection compared to in the control condition. But when the birds were housed in groups, this difference was abolished (Lopes et al., 2012). The same research group similarly showed that male zebra finches injected with LPS decreased their resting time when a novel female was added to their cage, compared to when alone (Lopes et al., 2013, 2023). These examples show that sickness behavior is not consistent across time and individuals. Instead, animals are constantly influenced by external factors, and may suppress their sickness behavior if needed, e.g., to take care of kin (Aubert et al., 1997) or if there is a mating opportunity (Lopes et al., 2013). Modulation of sickness behavior has not yet been studied experimentally in humans, but a recent study reported that humans can sometimes conceal sickness symptoms in different social situations (Merrell et al., 2024). Possibly, sick animals will only fully *embrace* their sickness behavior when the context allows for it. Such functional flexibility argues for a view of sickness behavior as motivated behavior, which competes with other motivational drives (Dantzer & Kelley, 2007).

1.3.3 Overt sickness behavior

Sickness will not only affect how you interact with others, but also how others interact with you (Dantzer, 2021). As described in the part on proactive behavioral defenses, sick individuals exhibit changes in their appearance and behavior that can be detected by others, and such *overt changes* have been argued to be part of sickness behavior since it may affect social interactions during sickness (Lasselin, 2021). For instance, sick animals move less (Hart, 1988), and sick humans have a gait profile characterized by a rigid and slow walking pattern (Lasselin, Sundelin, et al., 2020). In humans, there are also inflammation-induced changes in facial appearance, such as pale skin and lips, droopy mouth corners, and red eyes (see **Figure 4**). The sounds of the sick individual may also communicate their ill-health to others. Studies using the model of experimental endotoxemia show an increased level of yawns and sighs/deep breaths in sick individuals, compared to when healthy (Lasselin et al., 2018; Marraffa et al., 2017). Interestingly, the increase in sighs/deep breaths was only observed in male participants in that study (Lasselin et al., 2018), thus highlighting how overt sickness behavior may vary in different individuals and in different contexts. Altogether, this body of literature shows that sick individuals may look different to the world, but how does the world look to the sick?

Figure 4. Participants after an injection of saline and after an injection of lipopolysaccharide. Participants photographed two hours after receiving an injection of saline (blue) or an injection of lipopolysaccharide (orange). Participants were asked to keep a neutral facial expression. The individuals in the figure have provided consent for having their photographs in publications. Credit: Julie Lasselin.

1.4 Towards a better understanding of how sick individuals perceive sickness-relevant stimuli

In the framework of sickness behavior as an adaptive motivational state, we can suspect that sick individuals' perception of their surroundings will be colored by the benefit of keeping away or keeping close to specific stimuli. For example, a social interaction partner may be perceived differently depending on their likelihood to cause harm or provide care. Such perspectives are poorly investigated, and thus little is known about how the sick individual perceives the world.

1.4.1 Sickness behavior and perception of negative stimuli

For obvious reasons, measurements of sickness behavior in non-human animals are restricted to observation of behavior, while the same concept in humans includes the study of the additional dimension of *feelings* (Lasselin, et al., 2020a). Studies have shown an inflammation-induced increase in state anxiety (Lasselin et al., 2016) and negative mood (Harrison et al., 2009). The emotional state during sickness is also reflected in how negative stimuli are perceived. A study using the typhoid vaccine model found an inflammation-induced increase in sensitivity towards punishment (Harrison et al., 2016), a finding which was recently replicated using the LPS model (De Marco et al., 2023). When participants had to press a key as fast as possible in response to emotional stimuli (a Go/No-go task), sick individuals took longer time to process negative stimuli, compared to when healthy (Benson et al., 2017). Furthermore, sick individuals exhibited an increased sensitivity to negative social stimuli, and this sensitivity has been linked to inflammation-induced changes in brain regions important for emotional processing. For example, the increased negative mood observed after a typhoid vaccination correlated with the activity in the anterior cingulate cortex while looking at emotional faces (Harrison et al., 2009). In another study, participants recorded an interview and received feedback on their performance during a brain scanning session. Results showed that participant injected with LPS compared to participants injected with saline, had a higher activity in the amygdala and the anterior cingulate cortex, two brain regions important for detection of threats, when receiving negative feedback from an evaluator (Muscatell et al., 2016). Altogether, these findings indicate an inflammation-induced "negative bias" similarly to what is observed in depression (Dooley et al., 2018). Importantly, there is a well-established link between inflammation and depression, evident by the overlap between sickness behavior and depressive symptoms, as well as similar modulations of underlying brain functions (Dantzer et al., 2008; Dooley et al., 2018; Harrison et al., 2016). Hence, inflammation has been suggested to be one factor that could contribute to the "negative bias" observed in depression (Dooley et al., 2018).

The studies cited above investigating perception of negative stimuli have used negative words (Benson et al., 2017), learning tasks of punishment associated with abstract stimuli (De Marco et al., 2023; Harrison et al., 2016), and negative social stimuli such as fearful faces and negative feedback (Harrison et al., 2009; Muscatell et al., 2016). Yet, these studies do not capture how disgust stimuli, a type of threatening stimuli extremely relevant for the disease

state, is processed by sick individuals. Since disgust is part of proactive behavioral defenses against disease (see part 1.1.2), disgust stimuli could possibly be especially relevant for sick individuals who are already fighting an infection. Additionally, none of these studies investigated how inflammation affects cognitive reappraisal abilities. Cognitive reappraisal is a strategy for emotional regulation in which the goal is to change the meaning of a stimulus, for example by thinking that a threatening scene is only fiction (Gross, 2002). Given that inflammation affects brain regions that are central for emotion regulation (Buhle et al., 2014; Harrison, 2017), and that depressed patients exhibit a decreased ability to regulate their emotions (Erk et al., 2010), this is clearly missing when investigating how inflammation affects perception and processing of negative information.

To answer these remaining questions, we conducted **Study I** of the thesis, in which participants took part in a cognitive reappraisal task, with both general negative and disgust stimuli, after either receiving an injection of LPS or saline (see part 3.2.1).

1.4.2 Sickness behavior and perception of threatening social stimuli

Given the vulnerable state of sickness, it is sensible for a sick animal to try to stay away from further harm. Hence, it has been suggested that social withdrawal during sickness could be a strategy to avoid possible encounters with malicious others (Hart, 1988; Leschak & Eisenberger, 2019; Maier & Watkins, 1998). This is further evident in humans by an increased sensitivity to threatening social stimuli during acute sickness. For instance, when presented with different types of stimuli during brain scanning, participants injected with LPS had a greater activity in their amygdala while watching fearful faces compared to other types of pictures (e.g., non-social threatening images), but this difference was not found for participants injected with saline (Inagaki et al., 2012). There is also evidence for inflammation-induced avoidance of unfamiliar others. In a recent study, participants watched photos of close others and strangers (celebrities), and were asked to move a manikin towards or away from such stimuli, after receiving a vaccine against influenza. The authors found an association between the vaccine-induced increase in IL-6 concentration and more avoidance of strangers (Jolink et al., 2022). It is not known if other characteristics than the relatedness of the interaction partner affects how the sick individual perceives the individual. To speculate, contagious others may be threatening by carrying an additional pathogen, which could infect the already sick body. Yet, to be able to avoid contagious others, they first need to be detected as sick (Schaller & Park, 2011).

As described earlier, humans are able to identify sick individuals above chance, both from facial appearance (Arshamian et al., 2021; Axelsson et al., 2018; Leung et al., 2023; Tognetti, et al., 2023a), and from body odors (Olsson et al., 2014; Tognetti, et al., 2023b). Sick individuals also walk differently compared to when healthy (Lasselin, Sundelin, et al., 2020), and this change could thus be an additional cue for sickness. In a pilot study conducted by our research group, naïve raters where able to identify individuals injected with LPS from the way they walked on video recordings (Sundelin et al., 2015). Yet, the face of the walker was visible in the video clips (although small), and it is thus possible that the raters used facial

appearance as an additional cue when rating the stimuli. Point-light displays (PLDs) can be used to isolate perception of biological motion and posture from other cues. Imagine that you would put small lamps on each of your joints and then move in a completely dark room. Your movement would then be displayed with only a couple of moving points, i.e., with a *point-light display* (Johansson, 1973). Previous research has shown that humans are good at detecting emotions from PLDs (Dittrich et al., 1996), but it is not known if the state of sickness also can be detected.

In **Study II** of the thesis, we explored this notion by using recordings of walking individuals injected with either LPS or saline to create PLDs, along with video clips with blurred faces. We then presented these stimuli to healthy naïve raters in a sickness detection task in two separate sub-studies (see part 3.2.2).

As discussed above, sick individuals are in a vulnerable state and it may thus be even more important to detect and avoid contagious others when sick, compared to in a healthy state. Some findings illustrate that the characteristics of the observer can affect their ability to detect sickness in others. For instance, one study indicates that women are better than men at discriminating between sick and healthy faces (Tognetti, et al., 2023b). Furthermore, individuals with a vulnerable immune status appear to be more avoidant of pathogen cues (Miller & Maner, 2011). Yet, it is not known if the ability to detect sick others is affected by acute inflammation in the observer. To answer this question, we conducted **Study III** of the thesis, in which we let participants perform a sickness detection task, both after an injection of LPS and in a healthy condition (see part 3.2.2).

1.4.3 Sickness behavior and perception of safe social stimuli

Think about the last time you had a cold or a flu. You probably did not long for a dinner party or a blind date. Yet, maybe there was a specific person, such as a partner, parent or friend, who you longed for. Someone who could give you a cup of tea or a soothing hug. Indeed, inflammation-induced changes in social behavior entails an increased willingness to approach certain individuals (Leschak & Eisenberger, 2019; Muscatell & Inagaki, 2021), and this has been observed across taxa. For instance, apes cling more to their cage mates when sick compared to when healthy (Willette et al., 2007). In rats, an increase in huddling behavior has been observed after an injection of LPS, although such behavior could also reflect a strategy to increase body temperature (Yee & Prendergast, 2010). In an experimental setting that allowed rats to move between three cage compartments, sick rats spent the same amount of time in the social cage as the healthy rats. Yet, the sick rats also spent more time in the cage compartment furthest away from their cage mates, compared to healthy rats (Yee & Prendergast, 2012). These findings highlight that social sickness behavior is not all about social withdrawal, but neither all about social approach. Instead, the direction of the behavior seems to be influenced by several factors, such as kinship and the benefit of the interaction (Stockmaier et al., 2020).

In humans, there is some evidence for increased approach towards others during sickness. Remember the task in which participants completed an interview and received feedback in the scanner? In addition to being more sensitive to receiving negative feedback from the evaluator, participants injected with LPS were also more sensitive to positive feedback, as compared to the participants injected with saline (Muscatell et al., 2016). In another study, participants injected with LPS expressed an increased desire to be near a support figure (e.g., partner or parent), compared to healthy participants. There was also an LPS-induced increase in activity of the ventral striatum, a brain region important for reward processing, while watching pictures of support figures (Inagaki et al., 2015). In addition to these studies using the model of experimental endotoxemia, a recent study showed a positive relationship between IL-6 concentrations and the willingness to be near close others (Jolink et al., 2024).

These previous studies that investigated inflammation-induced approach behavior have focused on the relatedness of the social interaction partner (e.g., close others vs. unfamiliar others) (Inagaki et al., 2015; Jolink et al., 2022). But are all *unfamiliar others* perceived in the same way by the sick individual? What if they could provide care? Indeed, "stranger care" exist across human societies (e.g., by healthcare professionals and healers) (Kessler & Aunger, 2022). It is thus possible that sick individuals are willing to approach unfamiliar others, *if* they are perceived as especially inclined to provide care. In order to investigate how perception of caregivers is affected by the state of acute sickness, we conducted **Study IV** of the thesis, in which participants performed a caregiver perception task after receiving an injection of LPS and saline in a within-subject design (see part 3.2.3).

2 Aim

The overall aim of the thesis was to investigate perception of sickness-relevant stimuli and how such perception is modulated during immune activation. The model of experimental endotoxemia was used to pursue the following specific aims:

- To investigate the effect of experimental endotoxemia on cognitive reappraisal of emotions in response to general negative stimuli and disgust stimuli (**Study I**).

- To investigate if sickness, triggered using the model of experimental endotoxemia, can be detected from biological motion by naïve observers; and whether inflammation-induced differences in biological motion and sickness responses in the walker predict such detection (**Study II**).

- To investigate the effect of experimental endotoxemia on the ability to identify sick individuals from facial appearance and walking patterns; and whether sickness responses in the observer predict this ability (**Study III**).

- To investigate the effect of experimental endotoxemia on perception of caregivers (**Study IV**).

3 Methods

The model of experimental endotoxemia consists in intravenously injecting a small dose of the bacterial fragment LPS into healthy volunteers (see part 1.3.1). This model was used in **Study I**, **III**, and **IV** to make the participants experimentally sick. Furthermore, stimuli that were obtained from the participants in **Study I** were used in the sickness detection tasks in **Studies II-III**. All four studies thus comprise either behavioral assessments after an LPS injection and/or perception of individuals injected with LPS. Yet, these data collections have several important differences, such as the dose of LPS and general set-up. I will first describe the study design and sample for each study. I will then describe the experimental tasks used in each study. Lastly, I will summarize the measurements of sickness responses used as predictors for detection of sickness and LPS-induced behavioral changes. See **Table 1** for an overview.

3.1 Participants and overall study design

Study I was part of a double-blind, placebo-controlled, randomized cross-over study conducted in 2015 at Danderyd Hospital, Stockholm, Sweden. This was before I started my PhD, and I was thus not an investigator in the data collection, but I conducted the data analyses for **Study I**. The purpose of the study was to investigate how the administration of LPS affects behavioral outcomes, and the immunological basis of such changes (pre-registered at ClinicalTrials.gov: NCT02529592). To be eligible for the study, participants had to be between 18-50 years of age, without any somatic or psychiatric diseases, non-smokers, and not underweight or obese (see Lasselin et al., 2017). Twenty-two healthy participants (9 women; average age: 23±4 years) were included in the study and received an intravenous injection of LPS (*Escherichia coli* endotoxin, Lot HOK354, CAT number 1235503, United States Pharmacopeia, Rockville, MD, USA) at 2.0 ng/kg body weight, and an injection of saline (0.9% NaCl), at two occasions with a wash-out period of 3-4 weeks. The dose of 2.0 ng/kg body weight is considered a relatively high dose, giving rise to a strong inflammatory response and subsequent flu-like symptoms. Yet, there are still substantial variations in the response, with some individuals experiencing only mild sickness symptoms (Lasselin, 2021). Participants performed the cognitive reappraisal task (see part 3.2.1) during the second study day. Hence, **Study I** had a between-subject design even if the overall data collection had a within-subject design. One participant did not take part in the task, and the sample for **Study I** was thus ten participants injected with LPS and eleven participants injected with saline. Participants in **Study I** were photographed two hours post-injection and were then recorded during a walking task using a GoPro® camera as well as a Kinect® camera. These photos and recordings were used in the sickness detection tasks developed for **Studies II-III** (see part 3.2.2).

Study II consisted of two sub-studies for which participants were recruited to perform a sickness detection task (see part 3.2.2) in which they rated the stimuli obtained from the participants in **Study I**. For both sub-studies, participants had to be 18 years of age or older, and speak Swedish or English fluently. A sample of 106 participants was recruited for each

sub-study (sub-study 1: 70 women, average age: 30 ± 9 years; sub-study 2: 57 women, average age: 29 ± 8 years).

Study III was part of a data collection with a single-blind, randomized, and mixed between-subject/within-subject design. The study was conducted during the autumn of 2023 in the Sleep Lab at the Department of Psychology, Stockholm University, Sweden. The purpose of the main study was to explore how the behavior of the caregiver affected health outcomes after an LPS injection (pre-registered on OSF: 10.17605/OSF.IO/ZJ285). Thus, participants were randomized to two different caregiver behaviors ("augmented" vs. "limited" behavior), but this investigation is outside the scope of the present thesis. According to the inclusion criteria for the study, participants had to be 18-35 years of age, have a body mass index (BMI) in the range of 18.5-28 kg/m^2, and be completely healthy (without any diseases or ongoing medications). In addition, eligible participants were not allowed to smoke, use "snus", or drink excessively, and they had to be vaccinated against COVID-19. Thirty-five participants (18 women, average age: 26±5 years) took part in the study. All participants received an intravenous injection of LPS (Endotoxin Reference Standard, 10,000 USP Endotoxin Units, lyophilized, Catalogue #1235503, Lot HOK354) at 1.0 ng/kg body weight. The dose used in this study was lower than in **Study I**, yet slightly higher than the dose of 0.8 ng/kg body weight, which has been shown to provide strong variations in inflammatory response and sickness symptoms (Lasselin, 2021). Participants were also invited to take part in an extra visit to perform some of the tasks in a healthy state, including the sickness detection task (see part 3.2.2). Hence, **Study III** had a within-subject design, with an LPS condition and a control condition (no injection). Thirty-one participants took part in both the study day and the extra visit, while four participants only took part in one of the sessions (study day: N=3, extra visit: N=1).

Study IV was part of a double-blind, placebo-controlled, randomized cross-over study conducted in 2021-2022 at the MR centrum of Karolinska Institutet, Solna, Sweden. The purpose of the study was to investigate brain and psychological predictors of individual differences in the response to the administration of LPS (pre-registered on OSF: 10.17605/OSF.IO/MGU73). To be eligible, participants had to be 18-35 years of age, normal weight (BMI = 18.5-25 kg/m^2), and without any diseases or ongoing medication. The study took place during the COVID-19 pandemic, and all participants had to be vaccinated against COVID-19 and not have had confirmed COVID-19 or symptoms indicating COVID-19 six months prior to participation. Twenty-six participants were included in the study (15 women, average age: 25±5 years), but three participants developed COVID-19 before the second study day and could only take part in one session (LPS: N=1, saline: N=2). Participants received an intravenous injection of LPS (*Escherichia coli* endotoxin, Lot H0K354, CAT number 1235503, United States Pharmacopeia, Rockville, MD, USA) at 0.8 ng/kg body weight and an injection of saline (0.9% NaCl), at two occasions with a wash-out period of at least four weeks. Given that the purpose of the main data collection was to investigate individual-differences, the dose of 0.8 ng/kg body weight was selected to allow for inter-individual variations in sickness responses (Lasselin, 2021).

3.2 Experimental tasks

All studies (**I-IV**) rely on results from experimental computerized tasks, in which the participants watched and responded to different types of stimuli. See **Table 1** for an overview of the time point, stimuli, and response type of each study.

3.2.1 Cognitive reappraisal task

To investigate if experimental endotoxemia affected cognitive reappraisal of negative and disgust stimuli, participants in **Study I** performed a cognitive reappraisal task 4-5 hours after an injection of either LPS (2.0 ng/kg body weight) or saline. In the task, participants watched general negative stimuli (e.g., weapon) and disgust stimuli (e.g., vomit), and were asked to either *up-regulate* or *down-regulate* their emotions in response to the stimulus. A large amount of the stimuli were social, i.e., approximately 50% of the pictures included a face. Participants were trained upon inclusion and were provided with several strategies for cognitive reappraisal. For example, the investigator suggested, during training, that the participant could imagine that they themselves were in the picture (up-regulation), or imagine that it was only fiction (down-regulation). For each trial, participants had a fixation time, received the instruction (up-regulation or down-regulation), regulated their emotions in response to a stimulus (general negative or disgust), and rated their success in following the instruction from 1 (did not work at all) to 7 (worked very well) (see **Figure 5**). There was an unlimited response time. The task lasted approximately 13 minutes and consisted of two blocks with 26 trials in each (randomized instructions and stimuli), and a one-minute break between blocks.

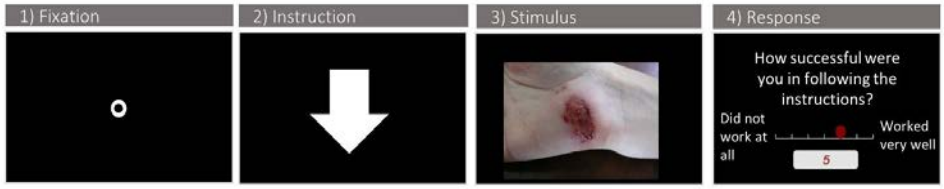

Figure 5. The cognitive reappraisal task in Study I. Each trial was organized as follows: (1) fixation time, (2) instruction to either down-regulate (arrow down) or up-regulate (arrow up) emotions in response to the stimulus, (3) presentation of the stimulus, consisting of either a general negative stimulus or a disgust stimulus (pictures), (4) rating of success in following the instruction from 1 (did not work at all) to 7 (worked very well).

3.2.2 Sickness detection tasks

Stimuli from participants in **Study I** (photos of faces, and video recordings and PLDs when walking) were used in the sickness detection tasks in **Studies II-III**. Participants' faces were photographed in a standardized setting two hours post-injection. The photos were cropped to control for different hair styles between study days and to hide sickness cues from the hair (e.g., messy hair while sick). A walking task was conducted directly following the photo session (2-2.5h post-injection). During the walking task, participants walked back and forth in

front of a GoPro® camera and a Kinect® camera. For the video clips captured with the GoPro®, one series (one back and forth) was selected and cut to only show the walk from the starting point (5.5m from the camera) to the camera. The face of each walker was blurred to hide facial cues. The Kinect® camera captures motion data by recording 3D coordinates for the body's joints at every time frame, and these data were used to create PLDs (see paper II for details on stimuli processing). Hence, from **Study I** we obtained stimuli (photos, video clips, PLDs) from individuals in a sick condition (injected with LPS at 2 ng/kg body weight) and from the same individuals in a healthy condition (injected with saline). Photos from all 22 participants were available for both conditions. Video recorded data were only available from 17 participants, and four additional PLDs were missing due to poor data quality (see paper II for details).

To determine if sickness can be detected from biological motion, participants in **Study II** watched and rated video clips of walkers with blurred faces, PLDs shown from the front (PLD0°), and PLDs shown from the side (PLD45°). In sub-study 1, participants watched the three stimulus classes separated in three blocks. The trials consisted of a fixation time, stimulus presentation, and a rating period. For each stimulus, the participant had to decide if the individual presented in the video clip or PLD was sick or healthy by pressing on the corresponding word on the screen. In sub-study 2, participants instead rated the health, tiredness, and sadness of each stimulus on a VAS (visual analogue scale) (see **Figure 6A**). The three ratings were separated in three main blocks, consisting of three sub-blocks with the three stimulus classes. The main blocks were separated with breaks of two minutes to avoid fatigue. The task for sub-study 1 lasted approximately 20 minutes, while the task for sub-study 2 lasted approximately 60 minutes.

To investigate the effects of experimental endotoxemia on sickness detection, participants in **Study III** performed a sickness detection task 2h15min after the injection of LPS, and during the control condition. In this sickness detection task, participants watched and rated photos of faces and video clips of walkers with blurred faces. The two stimulus classes were separated into two blocks with a one-minute break in-between. The structure of the trials was similar as for sub-study I of **Study II** (see **Figure 6B**). The task lasted approximately 15 minutes.

In all sickness detection tasks, the order of blocks was randomized, and the trials were randomized with the restriction that the same individual was not shown as sick and healthy in two trials directly following each other. In addition, participants had a five seconds response time limit to encourage spontaneous responses.

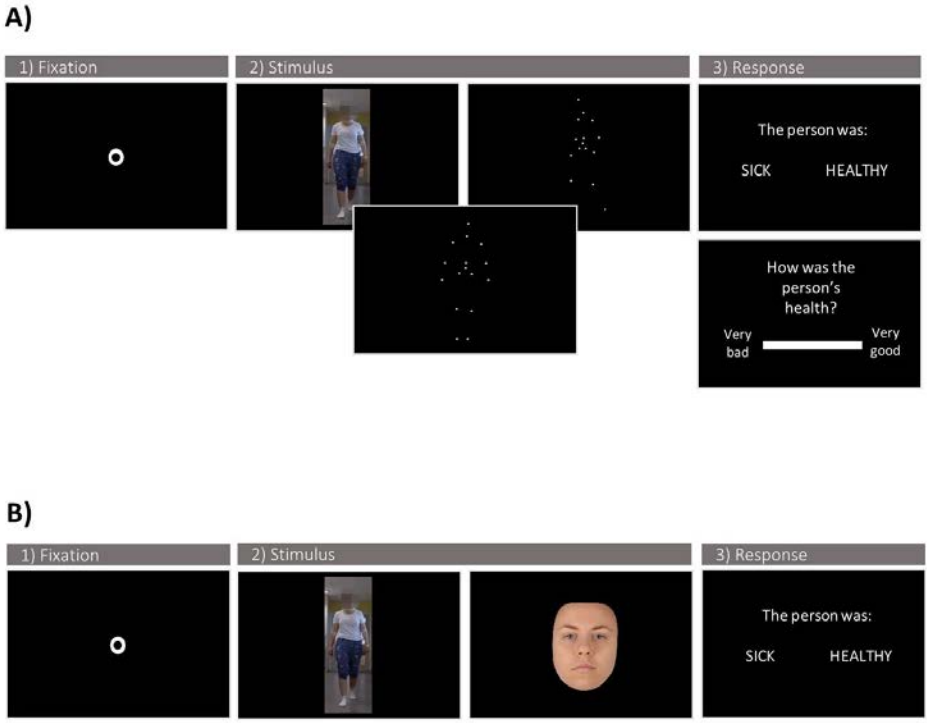

Figure 6. The sickness detection tasks in Study II (A) and Study III (B). Trials in both **Study II** and **Study III** consisted of a fixation time (1), stimulus presentation (2), and a reponse time (3). The stimulus classes were presented in separated blocks and consisted, in **Study II**, of walkers with blurred faces, point-light displays from the side, and point-light displays from the front (A2), and in **Study III**,of walkers with blurred faces and photos of faces (B2). In **Study II,** participants rated the stimuli as sick or healthy (sub-study 1) or rated the health, tiredness, and sadness of the stimulus on visual analogue scales (sub-study 2) (A3). Stimuli in **Study III** were rated as sick or healthy (B3). The visible person has agreed to have their photos in publications. The figure is adapted from a figure included in the draft of paper III.

3.2.3 Caregiver perception task

To investigate the effect of experimental endotoxemia on perception of caregivers, we developed a new task, the Caregiver Perception Task (CgPT). The task consisted of short video clips displaying three different types of interactions between a caregiver and a care receiver. Two of the video clip types showed a medical doctor and a sick individual, where the medical doctor was either taking care of the sick individual (e.g., providing water or helping the sick individual to stand up), or *not* taking care of the sick individual (e.g., working while the sick individual was reading). In the third type of video clip, the caregiver was a non-healthcare professional (i.e., a partner or parent) who was taking care of the sick individual (their partner or adult child). The video clips were recorded in the same context as the data collection with unprofessional "actors" portraying the different scenes (see paper IV for details).

Participants in **Study IV** performed the task 1h45min post-injection on both study days (i.e., both after the LPS injection and the saline injection). In the task, participants were presented with six video clips (two scenes per caregiver-care receiver interaction type) and had to rate the caregiver in each video clip according to likability, trustworthiness, and their willingness to interact with, and receive care from the caregiver. Additionally, medical doctors were rated on professionalism. All ratings were made on VAS scales (see **Figure 7**). Participants were video recorded during the task and these videos were later run with FaceReader 9 (Noldus, 2021) to analyze facial expressions.

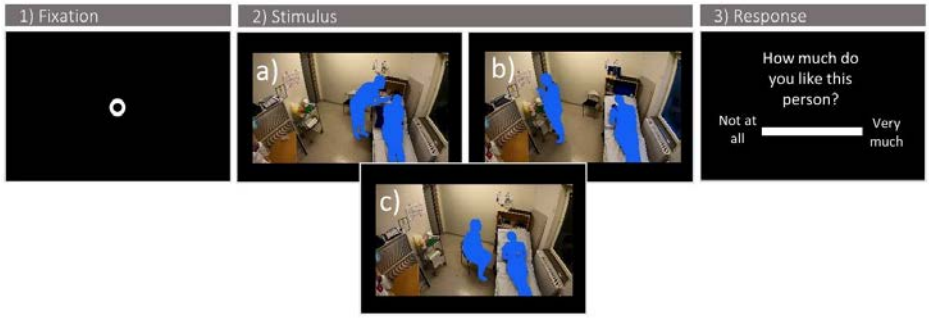

Figure 7. The Caregiver Perception Task (CgPT) in Study IV. Each trial was organized as follows: (1) fixation time; (2) presentation of a video clip of an interaction between a caregiver and a care receiver (the individuals in the figure are hidden for privacy), (3) rating of the caregiver on several aspects on visual analogue scales (the figure shows the scale for *likability*). The caregiver in each video clip was either a medical doctor providing care (2a), a medical doctor *not* providing care (2b), or a non-healthcare professional (e.g. partner, parent) providing care (2c). The figure is adapted from a figure included in the draft of paper IV.

3.3 Measurements of sickness responses

Measurements of sickness responses were used to 1) validate the LPS model in **Study I**, **III**, and **IV**, and 2) to investigate predictors of being detected as sick (**Study II**), or of LPS-associated changes in the ability to detect others as sick (**Study III**). The collection of such measurements will therefore briefly be described below.

3.3.1 Physiological inflammatory response and fever

Blood samples and vital parameters (including tympanic temperature, i.e., measured with an ear thermometer) were collected before the injection and regularly after the injection for participants in **Studies I**, **III**, and **IV**. In **Study I**, concentrations of IL-6, IL-8, and TNF-α were analyzed with high-sensitivity Luminex cytokine assays (Human Mag Luminex Performance Assay, LHSCM000, LHSCM206, LHSCM208, and LHSCM210, RnD Systems, MN, USA), while concentrations of IL-6, IL-8, IL-10, and TNF-α were analyzed with Meso Scale Discover assays (V-PLEX Custom Human Biomarkers assays, Meso Scale Discovery (MSD), Rockville, USA) in **Study IV**. Blood samples collected for participants in **Study III** are yet to be analyzed and are thus not included in the current work.

As predictors for detecting the individual as sick in **Study II**, we used the LPS-induced increase in IL-6 concentration and tympanic temperature from the measurements closest to the walking task in **Study I**. In **Study III**, The tympanic temperature from the time point closest to the sickness detection task during the LPS condition was used as a predictor for the ability to detect others as sick.

3.3.2 Subjective sickness responses

The sickness behavior of the participants in **Study I**, **III** and **IV** was measured during the study days with the Sickness Questionnaire (SicknessQ) (Andreasson et al., 2016). The questionnaire consists of ten statements which are rated on a scale from 0 (disagree) to 3 (agree) (see **Figure 8**).

		Disagree	Agree somewhat	Mostly agree	Agree
1.	I want to keep still	0	1	2	3
2.	My body feels sore	0	1	2	3
3.	I wish to be alone	0	1	2	3
4.	I don't wish to do anything at all	0	1	2	3
5.	I feel depressed	0	1	2	3
6.	I feel drained	0	1	2	3
7.	I feel nauseous	0	1	2	3
8.	I feel shaky	0	1	2	3
9.	I feel tired	0	1	2	3
10.	I have a headache	0	1	2	3

Figure 8. The Sickness Questionnaire (SicknessQ). The questionnaire (Andreasson et al., 2016) was used to assess sickness behavior for **Study I**, **III**, and **IV**. Scores on the questionnaire range from 0-30, where a higher score indicates more sickness behavior. The Swedish version was used in all studies, the English version is here shown for readability.

As predictors for detecting the individual as sick in **Study II**, we used the LPS-induced increase in sickness behavior, as well as a measurement of back pain on a VAS scale, closest to the walking task in **Study I**. In **Study III**, the measurement of sickness behavior closest to the sickness detection task in the LPS condition was used as a predictor for the ability to detect sickness in others.

Table 1. Summary of the methods of the studies included in the thesis

	Study I	**Study II**	**Study III**	**Study IV**
Title	Regulation of emotions during experimental endotoxemia: A pilot study.	The walking sick: Perception of experimental sickness from biological mortion.	Identifying sick people while sick yourself: a study of identification of facial cues and walking patterns of sick individuals during experimental endotoxemia.	Perception of unfamiliar caregivers during sickness – using the new Caregiver Perception Task (CgPT) during experimental endotoxemia.
Specific aims	To investigate the effect of experimental endotoxemia on cognitive reappraisal of emotions in response to general negative stimuli and disgust stimuli.	To investigate if sickness, triggered using the model of experimental endotoxemia, can be detected from biological motion by naïve observers; and whether inflammation-induced differences in biological motion and sickness responses in the walker predict such detection.	To investigate the effect of experimental endotoxemia on the ability to identify sick individuals from facial appearance and walking patterns; and whether sickness responses in the observer predict this ability.	To investigate the effect of experimental endotoxemia on perception of caregivers.
Participants	10 participants injected with LPS and 11 participants injected with saline.	Sub-study 1: 106 participants. Sub-study 2: 106 participants.	35 participants.	26 participants.
Design	Experimental, double-blind, placebo-controlled, randomized, between-subject design.	Experimental design.	Experimental, single-blind randomized, within-subject design.	Experimental, double-blind, placebo-controlled, randomized, within-subject design.
Dose of LPS	2.0 ng/kg body weight.	-	1.0 ng/kg body weight.	0.8 ng/kg body weight
Experimental task	Cognitive reappraisal task	Sickness detection task	Sickness detection task	Caregiver Perception Task (CgPT)
Time point of task	4-5 hours post-injection of saline or LPS.	-	2h and 15min post-injection of LPS.	1h and 45min post-injection of saline and LPS.
Stimuli	General negative and disgust stimuli (photos).	Video clips of walkers with blurred face, PLDs from the front, and PLDs from the side, from **Study I**.	Photos of faces and video clips of walkers with blurred face, from **Study I**.	Video clips of caregiver - care receiver interactions.
Ratings	Success in down-regulation/ up-regulation of emotions, from 1 (did not work at all) to 7 (worked very well).	Sub-study 1: rating of each stimulus as sick or healthy. Sub-study 2: health, tiredness and sadness (VAS scales).	Rating of each stimulus as sick or healthy.	Likability, trustworthiness, professionalism, and willingness to interact with and receive care from the caregiver (VAS scales).

Abbreviations: LPS: Lipopolysaccharide, PLDs: point-light display, VAS: visual analogue scale, ng/kg bw: nanogram/ kilogram body weight.

4 Main results

The aim of **Study I** was to investigate the effect of experimental endotoxemia on cognitive reappraisal of emotions. In accordance with an inflammation-induced "negative bias" (Benson et al., 2017; Harrison et al., 2009), we hypothesized that participants injected with LPS, in comparison with participants injected with saline, would report greater success in up-regulating their (negative) emotions, and less success in down-regulating their emotions. Moreover, we also hypothesized that this potential group difference would be stronger for disgust stimuli compared to general negative stimuli. When participants were instructed to up-regulate their emotions (i.e., to try to *feel more*) towards general negative and disgust stimuli, there was no difference between participants injected with LPS and participants injected with saline in reported success. Moreover, there was no difference in up-regulation of emotions towards general negative stimuli compared to disgust stimuli. Yet, when asked to down-regulate their emotions (i.e., to try to *feel less*) towards such stimuli, participants injected with LPS reported to achieve this *more successfully*, compared to participants injected with saline. In other words, participants who were sick felt that they could down-regulate their emotions towards general negative and disgust stimuli more easily than participants who were healthy, which was contrary to our hypothesis. We also found a significant main effect of type of stimuli, indicating that participants overall felt less successful when asked to down-regulate their emotions towards disgust stimuli, compared to general negative stimuli.

In **Study II**, we investigated if naïve observers were able to discriminate between sick individuals (injected with LPS) and the same individuals when healthy (injected with saline) based on their walking patterns. Our hypotheses were that naïve observers would rate sick walkers more often as sick, and as having worse health, compared to healthy walkers.
In sub-study 1, we found that participants were able to discriminate between sick and healthy walkers above chance level, both when watching video clips, and when solely observing the biological motion (i.e., from PLDs). In sub-study 2, sick walkers were rated as having significantly worse health compared to the same walkers in a healthy state, both when presented as video clips and PLDs (see **Figure 9**). In addition to looking less healthy, the sick walkers also looked significantly more sad and tired, compared to healthy walkers.

We also tried to characterize the *sick walk*, by investigating which specific LPS-induced changes in biological motion and posture predicted detection (sub-study 1) and health perception (sub-study 2) of sick walkers. The findings were different with regard to type of stimuli. For instance, in sub-study 1, only shorter steps predicted detection of sick walkers from video clips; but shorter, slower, wider, and more rigid steps predicted detection of sick walkers from PLDs. In sub-study 2, shorter, slower and more rigid steps predicted worse health ratings of sick walkers in video clips, while these parameters, in addition to more head-tilting downwards, predicted worse health ratings in PLDs. Another finding was that sick walkers in the video clips with a higher increase in IL-6 concentration in the LPS condition compared to in a healthy state, were rated as having worse health. Yet, the LPS-induced

increase in sickness behavior (SicknessQ), pain, and tympanic temperature of the walker did not predict sickness detection or health perception.

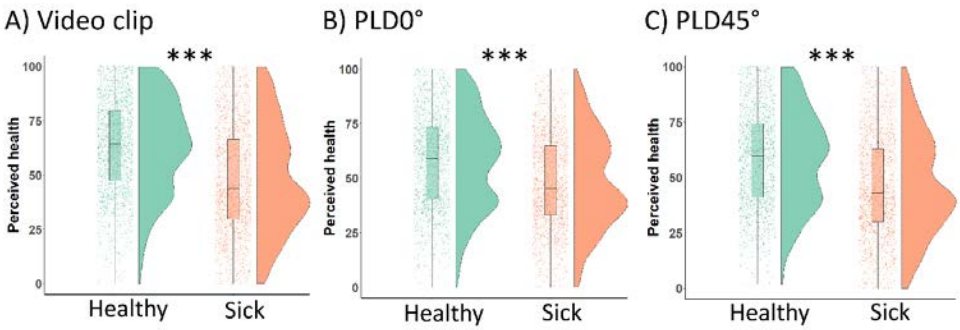

Figure 9. Health ratings of walkers in sub-study 2 of Study II. Health ratings of healthy (green) and sick (orange) walkers from video clips with blurred faces (A), PLDs shown from the front (B), and PLDs shown from the side (C). A higher score indicates perception of better health. Linear mixed models were used to assess the effect of walker condition (sick vs. healthy) on perceptions of health. ***p <.001. Abbreviation: PLD: Point-light display. The figure has been reproduced from Hansson, Lasselin et al. (2023). *Brain, Behavior, and Immunity*, 113, 319–327. doi: 10.1016/j.bbi.2023.07.020 (CC BY 4.0).

In **Study III**, we continued to investigate sickness detection abilities in humans by exploring if experimental endotoxemia affected such abilities. We hypothesized an LPS-induced overall increase in rating faces and walkers as sick. The results indicated that participants could detect sick individuals from their facial appearance and from walking patterns above chance level, both when they performed the task in the LPS condition and in the control condition. There was no significant difference between the LPS condition and the control condition in the ability to discriminate between sick and healthy *faces*. However, participants in the LPS condition rated more healthy *walkers* as sick, compared to in the control condition. In other words, participants were less good at discriminating between sick and healthy walkers when they were sick themselves compared to when they were healthy. The observers' sickness behavior (SicknessQ) and tympanic temperature while conducting the task after the LPS injection did not significantly predict the LPS-associated change in sickness detection ability (although power for this analysis was low).

The aim of **Study IV** was to investigate the effect of experimental endotoxemia on perception of caregivers. Our hypothesis was that participants injected with LPS, as compared to when injected with saline, would rate medical doctors providing care more positively compared to other caregivers. Additionally, we hypothesized an LPS-induced increase in facial expressions of happiness towards medical doctors providing care, compared to other caregivers. The results showed that the medical doctors who provided care to a sick individual were more positively rated on all aspects compared to the medical doctors who were not taking care of the sick individual (see **Figure 10**). Participants rated the medical doctors providing care and non-healthcare professionals providing care (e.g., a parent taking care of their adult sick child) equally positive, on several aspects, but medical doctors were significantly rated as more trustworthy compared to non-healthcare professionals (see **Figure 10b**). Additionally, participants showed an increased willingness to receive care from the medical doctors and non-healthcare professionals who provided care in the video clips in the LPS condition compared to the saline condition. This effect was not found for medical doctors not providing care (see **Figure 10e**). We did not find any differences in the participants' facial expressions while watching the video clips during the task between the LPS and saline conditions.

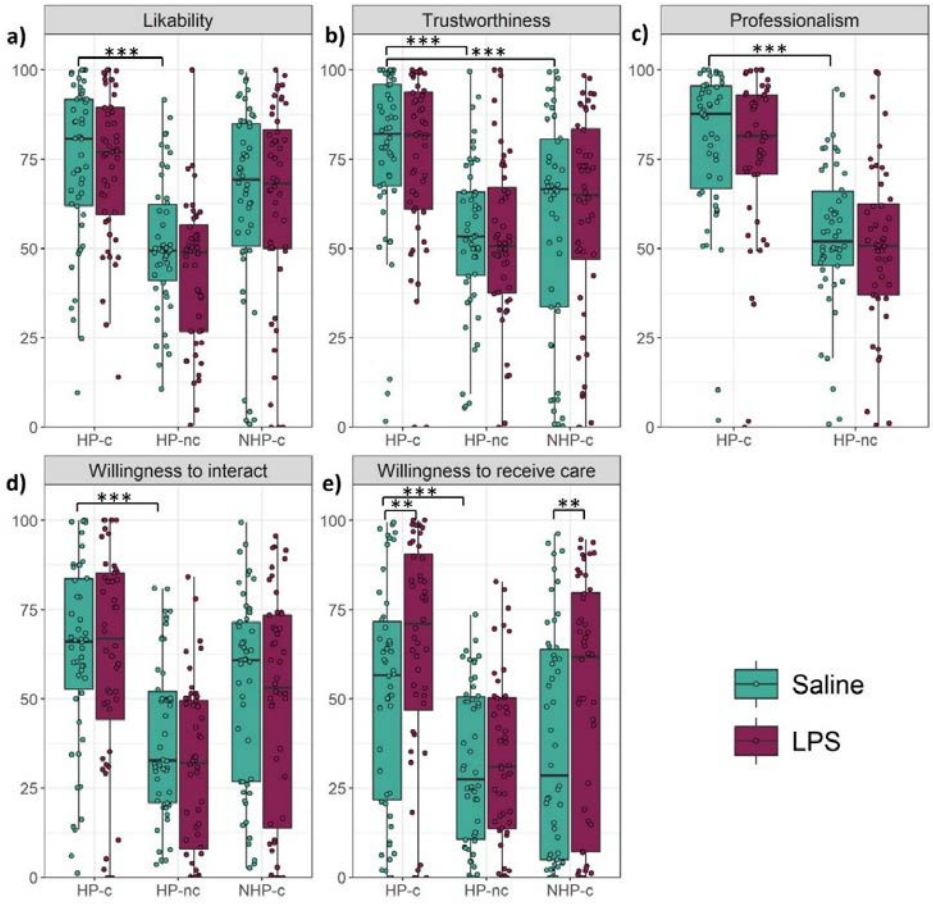

Figure 10. Ratings of caregivers in Study IV. Participants completed the Caregiver Perception Task (CgPT) once after receiving an injection of LPS (purple) and once after receiving an injection of saline (green). During the task, they watched video clips showing interactions between caregivers (medical doctor or non-healthcare professional) and sick individuals. There were three types of video clips: healthcare professionals, i.e. medical doctors, providing care (HP-c), medical doctors not providing care (HP-nc), and non-healthcare professionals (e.g., partner or parent) providing care (NHP-c). Participants rated each caregiver on several aspects: likability (a), trustworthiness (b), professionalism (c), willingness to interact with the caregiver (d), and willingness to receive care from the caregiver (e). Ratings were provided with visual analogue scales with higher score indicating more positive ratings. Linear mixed models were used to assess the effects of LPS and type of video clip (HP-nc, NHP-c) as well as the interaction effect (LPS*HP-nc, LPS*NHP-c) on ratings for each visual analogue scale. **p<.01,***p<.001. Abbreviation: LPS: lipopolysaccharide. The figure is included in the draft of paper IV.

5 Discussion

5.1 Summary of findings

In four studies, we investigated how humans perceive sickness-relevant stimuli, and how such perception is modified during immune activation. Results from **Study I** suggested that sick individuals felt more successful in down-regulating their emotions towards both general negative and disgust stimuli, compared to healthy individuals. In **Study II**, we demonstrated that naïve observers could detect sick individuals from the way they walk, and that such detection was predicted by specific inflammation-induced changes in biological motion and posture. **Study III** replicated findings from previous work (Andreasson et al., 2016; Arshamian et al., 2021; Sundelin et al., 2015; Tognetti, et al., 2023a) and from **Study II**, showing that sickness can be detected from facial appearance and walking patterns above chance level. Additionally, in **Study III**, we showed that individuals who were sick themselves could detect others as sick, but that immune activation modulated the ability to discriminate between sick and healthy walkers by increasing the number of false alarms (i.e. detecting healthy walkers as sick). Results from **Study IV** indicated that sick individuals, compared to when healthy, were more willing to receive care from both unfamiliar medical doctors and unfamiliar non-healthcare professionals who they had seen provide care to a sick individual in a video clip. An additional finding was that medical doctors who did not provide care in the video clips were perceived more negatively on all measured aspects (e.g., less likable and trustworthy) compared to both medical doctors and non-healthcare professionals providing care, and that participants were not particularly willing to receive care from such individuals both when healthy and sick.

5.2 General discussion

5.2.1 Social sickness behavior

All studies in this thesis relate to social sickness behavior. From how sick individuals are perceived by others (**Studies II-III**), to how sick individuals can regulate their emotions towards unpleasant (also social) stimuli (**Study I**), to how sick individuals perceive others and how this may be regulated by characteristics of the possible social interaction partner (**Studies III-IV**). Overall, the findings support the notion that inflammation affects social behavior, and that such effects are ambivalent and context dependent (Muscatell, 2021; Muscatell & Inagaki, 2021).

Our findings from **Study I** are in discrepancy with previous studies indicating an increased sensitivity to negative stimuli (Eisenberger et al., 2009; Harrison et al., 2009, 2016; Muscatell et al., 2016) and disrupted processing of such stimuli (Benson et al., 2017) (i.e., a "negative bias"), during sickness. Instead of the hypothesized inflammation-induced decreased ability to down-regulate emotions towards unpleasant pictures, we found the opposite, i.e. an inflammation-induced increase in the self-rated ability to down-regulate emotions towards both general negative and disgust stimuli. Importantly, **Study I** was a pilot study with a small

sample size and a between-subject design, and the findings should thus be interpreted with caution while waiting for replications. The absence of an *impairment* in the ability to regulate emotions during sickness is however intriguing. Indeed, a "negative bias" as a hallmark of both depression and inflammation has been used as an argument for inflammation-associated depression (Dooley et al., 2018).

However, even if several studies indicate that sick individuals perceive parts of the world more negatively, other aspects of sickness behavior complicate this picture. It is easy to imagine that sickness turns the world grey, in which nothing appears appealing. Yet, if you think about the last time you were sick, maybe there was something that you wanted, something that was worth the effort of getting up from the sofa for. For some people (me included), this appealing thing could be *chocolate*, and the same goes for mice. In a study where mice were trained to either nose poke 10 times to receive chocolate pellets, or nose poke one time to receive classic grain pellets, an injection of LPS decreased nose poking overall but the proportion of earned chocolate pellets increased (Vichaya et al., 2014). In other words, the mice where more willing to put in the effort if chocolate was the outcome. In another study, mice were trained to nose poke for chocolate in a similar way, but were then exposed to a shift in reward where they received a lower number of chocolate pellets for the same amount of work. This shift in reward reduced the effort similarly in both mice injected with LPS and mice injected with saline, thus indicating that sick mice were not more sensitive to this negative shift in reward (Casaril et al., 2021).

How much humans are willing to work for chocolate during sickness is yet to be tested, although a personal observation is that many of our participants surely enjoyed the piece of chocolate that they received at the end of the study day. Instead of chocolate, tasks with monetary rewards have been used in humans. In one study, participants performed a task in which they had to press a button rather slowly with the index finger of their dominant hand (low effort) or pressing a button fast with the little finger of their non-dominant hand (high effort). The trials had different probabilities of winning and different amount of money as the potential reward. The results from this study showed that sick participants were more likely to select trials with high effort for a high reward, compared to when healthy, at least when it was worth the effort, i.e. when the probability of winning was high (Lasselin et al., 2017). Thus, similarly to the sick mice in the study described above, sick humans were willing to work hard, but only for something *appealing*. Additionally, as described in part 1.4.3, sick humans are not only more sensitive to *negative* stimuli, they are also more sensitive to some *positive* stimuli. For instance, participants injected with LPS expressed an increased willingness to be near a support figure, compared to participants injected with saline (Inagaki et al., 2015).

These mixed findings suggest that the world perceived by the sick individual is not a world simply colored by a more negative state of mind. The world does not become all grey when sick, and some rewarding stimuli remain rewarding, possibly even more rewarding than in a healthy state. As suggested by **Study I,** sick individuals may even be better, or at least believe that they are better, at suppressing negative emotions. Yet, results from **Study I** could

possibly also be interpreted in another way. If inflammation increases the sensitivity to negative stimuli (Eisenberger et al., 2009; Harrison et al., 2009; Muscatell et al., 2016) and punishment (Harrison et al., 2016), the increased subjective success in down-regulation of emotions could be interpreted as increased avoidance of negative emotions. In other words, sick individuals may be more sensitive to negative stimuli and thus also more motivated to try to *feel less*. If the inflammation-induced increased success in down-regulation of emotions towards unpleasant stimuli is replicated, future studies should further investigate the function of such behavior.

Our findings in **Study III** indicate that sick individuals, as when healthy, are able to detect sick others above chance level. Interestingly, we also found that an injection of LPS affected sickness detection while rating walkers (but not faces). Sick observers incorrectly rated healthy walkers more often as sick, compared to when these observers were healthy. These results can be seen in the light of the "smoke detector principle" (Schaller & Park, 2011; van Leeuwen et al., 2023; van Leeuwen & Petersen, 2018). Just like your smoke detector may go off when there is smoke but no fire, the *pathogen alarm* may go off when there is no such threat present. It is better to have to climb up and turn of the smoke detector an extra time than to miss the flames, right? It is thus possible that sick participants would have an even more *sensitive* "smoke detector" that would start to beep for more "false alarms", resulting in them rating healthy walkers more often as sick. It can be argued that it would be favorable for an individual who is sick, and vulnerable, to have such an easily triggered system, and thus more prominent disease avoidance. Importantly, even if we observed an increase in healthy walkers rated as sick by sick observers, they still rated walkers with a slightly conservative bias (i.e., bias towards rating individuals as healthy). This could be explained by the fact that some of the individuals presented as stimuli did not express strong detectable sickness cues. The stimuli were derived from other individuals who were injected with LPS at one occasion, and saline at another occasion. Indeed, even if the dose was high (2.0 ng/kg body weight), some participants did not have pronounced sickness symptoms (Lasselin, 2021). In any case, the findings in **Study III** provide some evidence for how changes in *proactive* behavioral defenses (detecting and avoiding pathogen threats) may also be part of the *reactive* behavioral defenses (sickness behavior), but more studies are needed to establish such a link.

In **Study IV**, we showed that sick individuals were more willing to receive care from unfamiliar care providers, compared to when healthy. This adds to previous studies which have focused on how the relatedness between the sick individual and other individuals modifies the decision to approach others rather than to withdraw from all social interactions (Inagaki et al., 2015; Jolink et al., 2022). Our findings show that that the perception of the individual's ability to provide care also may shape such decisions. Of note, these findings are built on subjective data and we cannot know that the ratings of caregivers would affect actual behavior. For this reason, we also collected objective data, i.e. facial expressions in response to the caregivers, with the hypothesis of an LPS-induced increase in facial expressions of happiness towards medical doctors providing care, compared to other caregivers. Yet, there was no difference in facial expressions while watching caregivers, between participants when

sick compared to when healthy. An informal observation from watching these video clips of the facial expressions is that our participants looked focused, and thus neutral, during the task. It is possible that watching the video clips without the task to rate the caregivers would have allowed for more facial expressions. Future studies could also measure approach behavior by body movements (e.g., leaning towards or away from stimuli). To establish if the inflammation-induced increased willingness to receive care from unfamiliar caregivers translate into approach behavior would be an important next step to better understand the social aspects of sickness behavior.

One interesting question for social sickness behavior is the intensity of the inflammatory response needed for such changes to occur. Previous studies have shown inflammation-induced behavioral changes also with very low levels of inflammation, induced by a vaccine shot. For instance, a small increase in IL-6 after a flu shot increased implicit approach behavior towards close others, but had no effect on self-reported willingness to engage in social interactions (Jolink et al., 2022). Hence, the latter study suggests that implicit social behavior may be affected before explicit social behavior. Thus, there may be a "dose dependent" effect of inflammation on social behavior, and some behavioral changes may only occur with a strong immune activation (Lindsay, 2022). A more pronounced increase in inflammation may thus be needed for the individual to experience a switch in social needs. In the framework of sickness behavior being an adaptive process allowing behaviors that are beneficial for fighting off a pathogen (Dantzer, 2001; Hart, 1988), it is possible that it only would be adaptive for individuals with strong sickness symptoms to approach unfamiliar others for care. For instance, individuals with less need for symptom relief may benefit from using the energy for fighting the pathogen, instead of using it for social interactions with unfamiliar others. Indeed, many of our participants experienced strong malaise while performing the Caregiver Perception Task, and this *I am sick* state of mind may have affected the perception of the caregivers. More work investigating social sickness behavior in different models and contexts are thus needed to understand how these behaviors are affected by the strength of the inflammatory response. Such clarifications may also be interesting when trying to understand disrupted social behavior in health conditions with low-grade inflammation (e.g., inflammation-associated depression).

5.2.2 From the other side

The work of this thesis has mostly focused on how sickness affects social behavior from the sick individual's perspective, but healthy individuals may also adapt their social behavior depending on the health status of the social interaction partner. In **Studies II-III**, we add to the current literature on sickness detection in humans by showing that sick individuals can be detected solely from biological motion (**Study II**), and that observers can detect others as sick both when sick themselves and when healthy (**Study III**). During the data collection for **Study II**, many participants expressed how unsure they felt while rating the PLDs, some of them even became frustrated and I had to encourage them: *"just do your best!"*. It was thus fascinating for me personally that our participants, on a group level, were able to discriminate

between sick and healthy walkers from PLDs without apparently being aware of it. Importantly, similarly to other studies with similar design (Andreasson et al., 2016; Arshamian et al., 2021; Gordon et al., 2023; Olsson et al., 2014; Sundelin et al., 2015; Tognetti, et al., 2023a), our results showed that humans are able to detect sick individuals from various sickness cues, but that they are far from perfect in this ability. Yet, the fact that the participants were able to detect sick individuals from PLDs, which includes very little data, still highlights the role of biological motion in the perception of health status.

One interesting question is how the combination of sickness cues would impact detection of sickness. When you are categorizing someone as sick in the real world, you have access to several cues. For instance, you may see that the person have a pale face (facial appearance), is walking slowly and with a slumped and rigid posture (biological motion), and hear a cough (auditory). Such multisensory strategies are argued to be favorable for sickness detection (Hebets & Papaj, 2005; Stein & Stanford, 2008). Indeed, when photos of faces and odors were accessed simultaneously in a sickness detection task, sick faces were less liked when combined with body odors from someone sick than when combined with body odors from someone healthy (Regenbogen et al., 2017). Studies in which different types of sickness cues are shown separately and in combination could thus be an important future direction for understanding how humans detect sick others.

Many studies have focused on the detection of sick individuals, but little is known about what happens after such detection. One recent study showed that adults, but also children, more often choose an healthy individual, as compared to the same individual when sick, as a table partner during an imaginary dinner (Leung et al., 2024). Yet, this study was also based on subjective ratings, and more studies are needed to investigate avoidance behavior *per se*. One design option for such a study is to use a whole-body approach avoidance task, in which participants are asked to take a step towards or away from a stimulus (Stins et al., 2011). The time to initiate the step and the size of the step can then be used as measurements for approach (e.g., fast and big step towards a stimulus) or avoidance (e.g., fast and big step away from a stimulus) behavior. In relation to the findings in **Study III**, such a task could provide important information on the function of the observed decreased ability to discriminate between sick and healthy individuals. If sick individuals would be more avoidant to sickness-relevant cues, compared to when healthy, this would be an argument for an inflammation-induced increase in prophylactic behaviors.

To avoid *everyone* may be the best strategy to avoid contagious others, but this strategy is not a valid option for animals living in social groups. Humans and other social animals have many benefits from interacting with each other. Another individual can be a potential mate or collaborator, and too much avoidance could thus result in many missing social opportunities. Thus, animals need to make a cost-benefit trade-off in each situation to decide to approach or to avoid others. One factor that has been proved to affect such decisions is kinship (Kessler et al., 2017). As an example, you may move away from an individual with cold symptoms on the bus, but you would (probably) not leave your home if a family member started to cough.

Indeed, several studies show that infected animals are avoided by unfamiliar others, but not by their close kin. For instance, mandrills continue to groom their siblings and offspring, even when they are infectious (Poirotte & Charpentier, 2020). Similarly, vampire bats decrease their grooming behavior towards sick non-kin, but mothers continue to groom their sick offspring (Stockmaier et al., 2020). Humans clearly also take care of their sick family members, but little is known about how the caregiving affects the caregiver's own immune response. Interestingly, some studies have shown that pathogen cues can trigger the immune system. In one study, participants watched either photos of sickness-relevant stimuli (e.g., someone sneezing) or general threatening stimuli (e.g., guns). Upon adding LPS to blood samples collected after the stimuli presentation, immune cells from the participants who had watched sickness-relevant stimuli produced more IL-6 as compared to participants who had watched other threatening stimuli (Schaller et al., 2010). Moreover, two recent studies indicate that disease-relevant videos can trigger the release of salivary antibodies (Keller et al., 2022, 2023). Odors may also activate the immune system; exposing participants to disgusting odors (e.g., sweat) resulted in release of the cytokine TNF-α in the saliva (Anja Juran et al., 2022). These findings suggest that detecting pathogen threats trigger a priming of the immune system, making it ready to act in case the pathogen enters the body. Since caregiving requires close contact between the caregiver and the sick individual, it is possible that the caregiver's immune system also is primed in such situations. Such an effect would be adaptive as it would prepare the caregiver's body for possible intruding pathogens.

5.2.3 Strengths and limitations

The present thesis is built upon the model of experimental endotoxemia. The model is used in all studies, either as the experimental model (**Study I**, **III**, and **IV**) or to obtain stimuli (**Studies II**-**III**). This model comes with both distinct advantages as well as disadvantages. A major strength is that it allows for an experimental and controlled approach, thus reducing the noise from uncontrollable factors that would be expected in natural sickness settings. In comparison to the vaccine models, the injection of LPS induces a stronger inflammatory response, allowing us to study more pronounced sickness behavior. Yet, a clear weakness of the model is the generalizability. The participants in **Study I**, **III**, and **IV**, were all young and healthy, and the data collections were conducted in an experimental setting. Hence, it is not clear whether the current findings could be translated to other populations (e.g., elderly), other settings (e.g., emergency care), and other natural diseases (e.g., viral infections and depression). Additionally, expression of sickness behavior is affected by cultural factors (Shattuck et al., 2020), and cross-cultural studies are thus needed to verify that the findings are generalizable to other cultural settings.

The model of experimental endotoxemia entails a unique setting where the participant is aware of symptoms being transient and also non-contagious, and are constantly attended to by a medical doctor. Given that the present body of knowledge highlights the importance of the context on expressions of sickness behavior, it is possible that this unique setting may have affected the participants' performance in the various tasks. For instance, it may have

decreased the feeling of "vulnerability" which might have implications for how the participants perceive threatening stimuli, as well as safety stimuli. Moreover, sickness behavior has been argued to be adaptive within groups by reducing social interactions and spread of disease (Shakhar & Shakhar, 2015). The knowledge of being non-contagious may therefore further influence social sickness behavior. Arguably, the usage of the model of experimental endotoxemia is suitable as a first step to study sickness behavior in a controlled environment, but future studies need to replicate the findings in other settings.

In all four studies, we have used experimental tasks, including the Caregiver Perception Task that we have developed. The road to the final version of the task is however full of small crossroads. How many trials? Which type of stimuli? How do we phrase the questions to the participants? While designing the tasks for **Studies II-IV**, I have, together with my supervisors, tried to make as reasonable decisions as possible. (It should also be noted, that I love to follow these bumpy, but oh so creative roads.) Yet, during these years, I have thought a lot about the third crossroad example: how do we phrase the questions? The goal of this thesis has been to investigate how stimuli are perceived. But what about how the participants are interpreting the question(s) on how they perceive the stimuli? In **Studies II-III**, participants watched video clips, PLDs, and photos of individuals injected with either LPS or saline, and we asked them if the person was *sick* or *healthy*. The word *sick* in this context is meant to capture if the participants are able to detect the individuals who are injected with LPS, and thus have an ongoing inflammation and in many cases, sickness symptoms. Yet, we do not know how the participants interpret the word. It is possible that some participants thought specifically about infectious diseases (such as a cold or flu), while rating the stimuli. Others may have perceived *sick* as a broader term, also including chronic diseases. For instance, when detecting sick individuals based on walking patterns, it is possible that some participants thought about chronic diseases known to affect movement, such as Parkinson's disease. In the framework of sickness detection as part of proactive behavioral defenses against disease (Schaller & Park, 2011), it may thus have been more sensible to use *contagious* and *not contagious*, to capture if the participants were perceiving the presented individuals as pathogen threats. Yet, according to the "smoke detector principle" described above (Schaller & Park, 2011; van Leeuwen et al., 2023; van Leeuwen & Petersen, 2018), humans may be sensitive to unspecific deviations that could, and could not, be a sign of contagiousness. This suggests that we may have had similar results from different questions, since the *sick* and *contagious* options are both representing detection of *something "abnormal"*. Indeed, after participants watched video clips of different individuals who touched items and then were asked to touch the items themselves, participants showed equivalent amounts of disgust and avoidance behavior to items touched by someone with a big birth mark and someone with flu-like symptoms (Ryan et al., 2012). Future research on sickness detection in humans would gain from designs that mix stimuli (e.g., from individuals with different types of diseases) and questions (e.g., sick vs. contagious) to further disentangle these matters.

In relation to the discussion in the previous paragraph, I believe that all experimental tasks used for the studies in the present thesis would have gained from follow-up open questions to the participants. For example, questions regarding specific emotion regulation strategies used by participants in **Study I**, and from where participants in **Study IV** thought the video clips were obtained would have helped in interpreting the findings, and for future experimental designs.

5.2.4 Ethical considerations

The main ethical consideration for the thesis is the usage of the model of experimental endotoxemia. The model is considered safe for studying sickness behavior in healthy volunteers, and have been used for 30 years with no long-term effects. Also, in studies with doses twice as large as used in the present studies (2.0-4.0 ng/kg body weight), only a small number of adverse events were reported (e.g., bradycardia/asystole). To reduce likelihood of adverse events, participants are thoroughly screened prior to inclusion to ensure that they are healthy.

Even with low risks for adverse events, it should be considered if it is ethically tenable to induce symptoms such as fever, headache, and nausea, in healthy volunteers. Indeed, as an investigator involved in two data collections with the model, I have encountered participants with strong symptoms, such as high fever and vomiting, even if the dose of LPS was relatively low (0.8-1.0 ng/kg body weight). Notably, participants are informed about possible stronger LPS-induced symptoms before providing consent. They are also constantly attended to by a medical doctor, who ensures their safety and helps them to relieve symptoms. An additional important point is that the symptoms are transient and vanish after 4-6 hours. All participants are aware of this aspect, and this knowledge may facilitate coping with such symptoms. Yet, if the symptoms are unbearable, or for any other reason, participants have the possibility to withdraw, and then receive an anti-inflammatory drug. The participants are also compensated for their time (e.g., 3500 SEK in **Study IV**). Lastly, most of our previous participants reported that they are willing to take part in a similar study again. For example, all participants in **Study IV** provided a rating between 3 (maybe) and 5 (absolutely) when asked about reparticipation. Given the importance of studying acute sickness in humans, and the possibility to translate findings to inflammation-associated conditions such as depression (Lasselin, et al., 2020b), the model can be considered ethically justifiable.

The data collections using the model of experimental endotoxemia entail the collection of sensitive data such as video recordings, photos, and biological material. All data were handled in accordance with GDPR, and participants were extensively informed about our data procedures and their rights before providing their consent. Participants also had the possibility to restrain from providing certain data (e.g., to not be recorded).

In the cognitive reappraisal task, participants watched negative stimuli which might have provoked feelings such as fear and disgust. Participants were aware of the possibility to stop

the task at any time point. The tasks for **Studies II-IV** did not include any stimuli suspected to induce strong negative emotions.

Ethical considerations also relate to the transparency and reproducibility of the present work. During my time as a PhD student, I have tried to follow open science practices to the best of my capacity. The studies using the model of experimental endotoxemia (**Study I**, **III**, and **IV**) were preregistered prior to the start of the data collections. Analysis plans were openly published via OSF before data collection (**Studies III-IV**), or prior to data analysis (**Study II**), and deviations from the plans were stated in the corresponding papers. Data and scripts for **Study I** and **IV** are similarly published via OSF, and can thus be reviewed and used for additional analyses. Unfortunately, due to the sensitive nature of the data in **Studies II-III**, and because they were collected before GDPR was established, we are not entitled to share the data according to GDPR. These circumstances are clearly described in each paper for transparency.

5.3 Future directions

Study IV moved social sickness behavior from static stimuli to dynamic stimuli. I believe that an important future direction is to go one step further, from dynamic stimuli to real life. Several studies have investigated how sick individuals perceive social stimuli, but little is known about how this translates into actual behavior during social interactions. In a recent study, humans reported that they may conceal their sickness symptoms in certain social contexts (Merrell et al., 2024). This notion is especially interesting in the light of animal models showing that sick animals can suppress their sickness behavior if needed (Aubert et al., 1997; Lopes et al., 2012). Hence, a next step could be to experimentally investigate how sick humans regulate their sickness behavior in different social settings. This relates especially to overt sickness behavior (e.g., changes in gait and sounds), which could possibly be modulated depending on the adaptiveness of concealing or not concealing. For instance, overt sickness behavior could be favorable in the presence of a potential caregiver, but less favorable in a context when the sick individual wants to hide their vulnerability. The model of experimental endotoxemia would be a good first step for such investigations, since it induces acute sickness with symptoms possible to conceal (i.e., no upper respiratory symptoms).

In addition to experimental models of sickness, it would be interesting to investigate how expressions of sickness in kin affects caregivers' sickness responses in families. Just like the sick mice dams suppress their sickness behavior while building a nest for their pups (Aubert et al., 1997), do parents (tend to) suppress (or *conceal* to themselves?) their sickness symptoms while taking care of a sick child?

5.4 Conclusions

With the four studies included in this thesis, we have added to the current knowledge on social sickness behavior from several angles. In relation to how sick individuals are perceived by others, we showed that humans are able to detect sick individuals solely from biological

motion and posture. Furthermore, we nuanced the view of an inflammation-induced increase in sensitivity to negative stimuli (Benson et al., 2017; Harrison et al., 2009), by suggesting that sick individuals report themselves as more successful in down-regulating their emotions towards threatening stimuli, as compared to healthy individuals. Furthermore, we showed that the ambivalence of social sickness behavior (Leschak & Eisenberger, 2019; Muscatell & Inagaki, 2021) is not merely explained by the relatedness of the social interaction partner. Instead, sick individuals may also be willing to approach unfamiliar individuals, if they are perceived as possible care providers. We also showed that sick individuals are worse at discriminating sick and healthy walkers, because of an increase in healthy walkers incorrectly rated as sick (increase in "false alarms"). Speculatively, this could indicate that proactive defense behaviors could be promoted during a vulnerable state of sickness. Altogether, these findings highlight the complexity of the perception of sickness-relevant stimuli, showing that sickness behavior is not all about a "negative bias", nor all about social withdrawal. The past has focused on the *how* and *why* of sickness behavior. I hope for a future of *where* and *when*.

6 The PhD journey

In some ways, this journey already began in 2017 when I started to work as a research assistant at Emotion lab (KI CNS) led by Andreas Olsson. During my time in the lab, I helped to collect data in many projects using fear conditioning paradigms (e.g., Espinosa et al., 2022; Undeger et al., 2020), and I also learned to collect and preprocess psychophysical data. It was when I saw all the creative approaches to studying social behavior in humans that I fell in love with the research world, and started to dream about starting a PhD.

In June of 2018, I was sitting with two colleagues at a summer party and suddenly said *"I want to study how the immune system affects social behavior"* and they said *"You know there is a research group at KI doing that right?"* Little did I know that by the end of the summer there would be an opening in this group, and two years later I would start my PhD.

In addition to what is presented in this thesis, I have been part of several projects which have contributed to my development into an independent researcher. First of all, I have developed and collected data for two tasks which are yet to be analyzed, including an Implicit Association Test (IAT) to measure positive bias toward healthcare professionals, and a whole body approach-avoidance task using a Kinect® camera to capture participants' movements in response to stimuli. I look forward to seeing how the findings from these tasks will add to the results presented in this thesis. Additionally, I have been involved in several side projects, including one study on how seasonal allergy affects behavior, and one study on health anxiety during the COVID-19 pandemic. For both of these projects, I helped to prepare and run the data collections. These side projects provided me with important knowledge in how to run studies in patients (allergy project), and how to prepare and run online studies (health anxiety project). I also learned important methodological skills, including how to conduct several pain tests using an algometer (allergy project). Together with my principal supervisor, I have contributed with data to a big international project on human social motivation during the COVID-19 pandemic (Pick, et al., 2022a, 2022b), and thus gained insight about the processes of such collaborations. Lastly, while helping to prepare and run one LPS study and being the study organizer of another LPS study, I have learned an endless amount of things related to how to design, organize, and manage such studies. Even if these studies were remarkably challenging, I am grateful for everything they gave me. I am now prepared for *anything*.

Altogether, this journey has been the perfect mix. A mix of immunology and psychology. Of small studies and big studies. Of designing, preparing, collecting, analyzing, and writing. In many ways, it was the perfect PhD journey. At least now, when I look back and can see the whole road. Yes, I can see it so clearly now. How it all led me here.

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