BACHELOR THESIS

The influence of Toxoplasma (T.) Gondii on the brain and behaviour

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Foreword

This thesis was commissioned by the pre-master Biomedical Sciences of the RUG. Interest in Neurological Biology was sparked by the course Neurobiology, part of the pre-master program. Subject of the thesis is inspired by the recent article of Kelly Servick in Science magazine: *Brain parasite may strip away rodents' fear of predators—not just of cats.*

Abstract

The protozoan parasite *Toxoplasma gondii (T. gondii)* is a single cell eukaryote that uses its host cells to survive. In order to survive it has three stages in life, tachyzoites, bradyzoites or sporozoites, with the goal to end op in definite feline hosts. Hosts are protected by initiate and secondary immunity against this parasite; however immune cells can get invaded by *T. gondii* where they are used to enter organs and the BBB. When the parasite is manifested in the host it can cause behavioural changes, in rodents specifically it can lead to fatal attraction towards the definite host. In psychiatric diseases such as Alzheimer's, Parkinson's and Schizophrenia but also depression and suicidal behaviour in humans *T. gondii* also seems to be of influence. Hard definite conclusions about the involvement of *T. gondii* in behavioural changes and psychiatric diseases are hard to make because of the many possible factors found and how poorly their underlying mechanisms are understood.

In this thesis

Introduction

The protozoan parasite *Toxoplasma gondii (T. gondii)* is a single cell eukaryote that uses its host cells to survive (J. A. Kochanowsky, 2018). *T. gondii* infects different kinds of mammalian species and infects up to a third of the world population (J. G. Montoya, 2004). Infection with *T. gondii* often has an influence on the eyes and/or brain causing seizures for example (S. Berger, 2019). The capability of *T. gondii* to infect the brain and the Central Nervous System (CNS) causes the association of the parasite with human neuropsychiatric conditions in humans. In rodents it has been seen that infection alters behaviour (S. Tyebji, 2019).

In fact, recently there was stated that the parasite may strip rodents of their fear for the predators (K. Servick, 2020). In the supporting article there was found that *T. gondii* infection lowers general anxiety, increases explorative behaviour and alters predator behaviour (M. Boillat e. a., 2020). This raises the question; how does this parasite influence the brain and its hosts behaviour?

Beginning answering this question it is important to know how *T. gondii* infects its host and in which way the immune system reacts. Secondly to understand how fear is influenced in these rodents it is important to know which brain part(s) are infected and how this has an influence on the behaviour. Lastly the focus can be shifted to human to see if there is evidence of correlation between toxoplasmosis and neuropsychiatric diseases.

Life cycle of *T. gondii*

To survive *T. gondii* has a complex life cycle that uses definite hosts (DHs) and intermediate hosts (IHs). The transmission between DHs and IHs of the parasite is manifested with the use of oocysts. The parasite has three stages that are important for survival, the tachyzoite is associated to active infection, the bradyzoite in tissues and the sporozoite which can survive in the environment (S. J. Hadfield, 2017). Cats, the DH, get infected by eating infected prey. After infection of the DHs, formation of oocysts takes place in the intestine and get excreted in faeces. The oocysts get sporulated outside the host, forming two sporocysts with four sporozoites (A. M. Tenter, 2000) (J. G. Montoya, 2004). The number of days before shedding of sporozoites depends on the oocysts ingested (J. P. Dubey, 2002). Ingestion of the virus by the DH results initiation of the sexual multiplication and ingestion by IHs initiates the asexual development (J. G. Montoya, 2004) (E. Gilot-Fromont, 2012).

In the first asexual phase rapidly multiplying tachyzoites are present in many different host cells (A. M. Tenter, 2000). Tachyzoites enter these host cells actively and form a parasitophorous vacuole (PV). Via the bloodstream the tachyzoites infect many tissues are mainly localized in the CNS but also in organs such as the brain, muscle, placenta, udders and gonads (J. G. Montoya, 2004) (E. Gilot-Fromont, 2012). The tachyzoites of the last generation activate under influence of immune system the second phase of asexual development (A. M. Tenter, 2000) (J. G. Montoya, 2004). In this phase tissue cysts, bradyzoites, are formed. Thousands of these bradyzoites form the tissue cyst in the brain or other organs (A. M. Tenter, 2000) (E. Gilot-Fromont, 2012). Infection of different organs give different transmission possibilities for the virus to be transmitted between IHs. Vertically via the placenta, pseudo-vertical via milk and sexually via sperm. But also, carnivory and blood/organ transplantation are routes of infection (E. Gilot-Fromont, 2012).

Immune response towards *T. gondii*

Human infection is caused by consuming sporozoite contaminated food (S. J. Hadfield, 2017). In many mammals the main route of infection of *T. gondii* is via oral ingestion. In this way it enters the intestinal tract where the parasite infects the epithelial cells (L. Kasper, 2004). *T. gondii* invade the host cell actively by using an actin-dependent myosin motor complex (V. B. Carruthers, 2002).

Innate immunity

The immune systems recognizes the pathogen-associated molecular patterns (PAMPs) of *T. gondii* presented by presenting cells such as dendritic cells (DCs) or macrophages with the use of toll-like receptors (TLRs) (M. I. Sasai, 2018) . The recognition and resistance of the host to pathogens such as *T. gondii* is dependent on the IL-12 production by dendritic cells (DCs), neutrophils and macrophages (S. K. Bliss, 2000). One important factor in the *T. gondii* induced IL-12 production is chemokine receptor CCR5, cyclophilin-18 of *T. gondii,* induces the expression of CCR5 in DCs (J. Alberti, 2000). MyD88-deficient mice models infected with *T. gondii* show a complete defect in acute resistance and impaired IL-12 production. Indicating that TLR recognition with help of these MyD88 and IL-12 factors are utterly important in the innate immunity against *T. gondii* (C. A. Scanga, 2002). *T. gondii* does not only induces IL-12 production by DCs and macrophages but also production of IL-1, TNF-α, CCL2, CXCL2 (M. I. Sasai, 2018).

Secondary immune response

IL-12 production triggers proliferation of NK cells, CD4 T cells and CD8 T cells, all produce high concentrations of IFN-γ. In secondary immune response neutrophils and monocytes are migrated to the *T. gondii* infection site by cytokines CCL2 and CXCL2. Monocytes produce IL-10 which is involved in protecting the brain against toxoplasmosis. (M. I. Sasai, 2018).

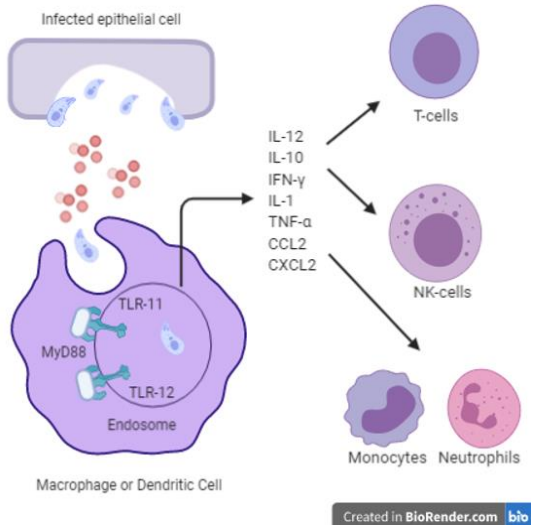

Figure 2: Host immune response to *T. gondii* infection. *T. Gondii* infected epithelial cells activate the immune response by macrophages and/or Dendritic cells. Via TLR-11 and TLR-12 with MyD88 complexes the cells produces cytokines and chemokines IL-12. IL-10, IFN-γ, IL-1, TNF-α, CCL2 and CXCL2. These activate T-cells, NK-cells, monocytes and neutrophils and guide them to site of infection.

Figure 1 is a summary of all the cytokines or chemokines that are produced when a cell is infected with *T. gondii*. The cytokines induce activation of T cells, NK-cells, monocytes and neutrophils. But cells are not only activated, chemokines help the movement of immune cells to site of infection. If parasites such as *T gondii* do enter the cell Nitric oxide synthase (NOS2) is important in the inhibition of the parasiticidal activity. The disruption of the formed PV is induced by immunity-related GTPases (IRGs), under influence of IRGM1-IRGM3 and ATG5. There are two families of IRGs, GKSs and GMSs. IRG (P47 GTPase) protein functioning is depended on underlying interactions between GKS-GMS (J. P. Hunn, 2008). Disruption of the PV is started with IFN-γ induced transcription of P47 GTPases Irgm1, Irgm2 and Irgm3 of the GMS family and ATG5. This induces expression of Irga6 and Irgb6 of the GKS family (J. P. Hunn, 2008) Not only P47 GTPases but also P65 guanylate-binding proteins (Gbps) GTPases get expressed in the PV disruption pathway (M. Yamamoto, 2012). In particular Gbp1 and Gpb2 are essential in the IFN-γ mediated defence against *T. gondii* (E. M. Selleck, 2013) (D. Degrandi, 2013). Figure 2 shows a summary of how the PV is disrupted and parasite activity is reduced by NOS2.

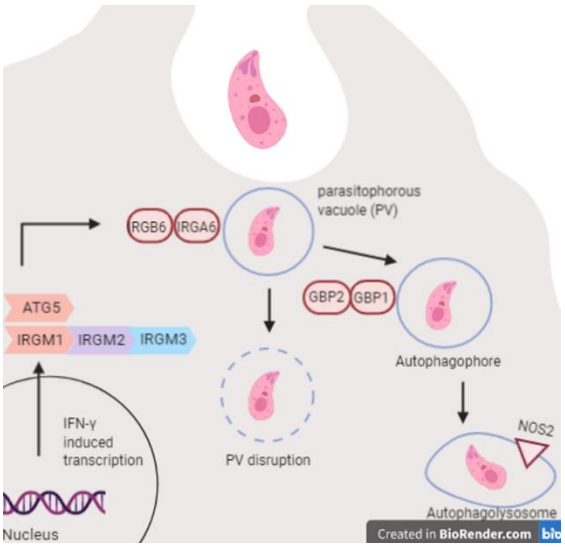

Figure 1: Parasitophorous vacuole (PV) disruption. Disruption of the *T. gondii* vacuole involves IFN-γ induced transcription of IRGM1, IRGM2, IRGM3 and ATG5. This activates IRGA6 and IRGB6 eventually causing PV disruption. GBP1 and GBP2 induces NSO2 inactivation of *T gondii*.

Even though the immune system works hard to get rid of *T. gondii*, the parasite can enter not only the intestine epithelial cells but also the immune cells such as leukocytes, dendritic cells and macrophages. In the disguise of an immune cell the parasite can reach many organs and even cross the blood-brain barrier (BBB). Even not in disguise of body's own cells the parasite can cross BBB through openings in tight junctions. Because *T. gondii* can enter host (epithelial) cells it is even thought that the parasite can enter the brain epithelial cells. Where it can be replicated and then releases into the functional tissues of the brain (A. Krishnan, 2016).

T. gondii influences the rodent brain and its behaviour

Because of the capability of *T. gondii* to cross the BBB and enter functional tissues of the brain it is reasonable to think that the parasite is capable of influencing brain function and behaviour. This is seen in rodents such as rats and mice, when infected with *T. gondii* they show change in behaviour towards their predators (M. Berdoy, 2000). It seems that the aversion towards cats is changed into a fatal attraction.

Changes in behaviour

Changes in behaviour after *T. gondii* infection can be approached by different types of behavioural tests. Locomotion and anxiety in an open-field arena, fear conditioning, neophobia towards food and hippocampal learning are for example not influenced by infection. But the aversion to cat odour shifts to attraction (A. Vyas, 2007). (P. K. House, 2011) Making it possible to assume that the shift of aversion toward cat odour to attraction is a specific change in behaviour induced by the parasite. However infected mice seem to show lower general anxiety and an increase in explorative behaviours (M. Boillat e. a., Predator Cat Odors Activate Sexual Arousal Pathways in Brains of Toxoplasma gondii Infected Rats., 2020). This could mean that the attraction towards cat odour is just a result of this lower anxiety and explorative behaviour.

Location of cyst in the brain

T. gondii is detected throughout the brain and there seem to be a few brain regions (cortical and subcortical) where most cysts are detected. This is possibly due to different factors such as the properties of brain-blood barrier and cytoarchitectural features locally, metabolism, blood flow and natural barrier for *T. gondii.* The location of cysts has different behavioural changes as result. Cysts in motor cortices lead to motor and coordination deficits. Cysts in visual cortex and olfactory bulb lead to sensory deficits. Cysts in the dorsal hippocampus leads to cognitive deficits. And cysts in the amygdala and ventral hippocampus lead to decreased aversion to predators. (M. Berenreiterova, 2011). Even though different regions are seen in the localization of the cysts there is evidence that there is a prevalence towards the amygdala region (A. Vyas, 2007). The number of cysts is positively correlated to the severity of the changes in behaviour (M. Boillat e. a., Predator Cat Odors Activate Sexual Arousal Pathways in Brains of Toxoplasma gondii Infected Rats., 2020). Most interest is sparked by the cysts in amygdala (and hippocampus) because of the possible prevalence for this region and the possible influence on behaviour towards the predators.

Changes in the brain that influence behaviour

The changes in behaviour seem to be influenced by *T. gondii* presence in the brain. As the parasite is found in different regions of the brain it is hard to pinpoint the exact mechanism behind the changes in behaviour. But it seems that infection with *T. gondii* affects neuronal function and structure (A. Parlog D. S., 2015). Many different researches have given theories with different kinds of influences on neuronal function and structure, there seem to be direct and indirect influences.

Looking at direct influences of *T. gondii* on the neuronal function or structures there are different theories. As mentioned before the location of cysts seem to have different influences in behavioural changes (M. Berenreiterova, 2011). Other theories involve the role of dopamine, silencing of neurons, and blocked apoptosis. Excess production of dopamine can interfere with crucial brain function such as cognition, memory, learning and mood and in this way influence behaviour (A. Parlog D. S., 2015). *T. gondii* processes two genes that encode tyrosine hydroxylase; that produces L-DOPA a precursor of dopamine (E. A. Gaskell, 2009). In the amygdala, involved with fear behaviour, dopamine and its metabolites are increased after infection with *T. gondii.* Specific cells producing dopamine show an increased level of dopamine after infection. The increased levels of dopamine possibly reduce the fear experienced by

infected individuals (F. Ihara, 2016). (E. Prandovsky, 2011). Another theory is the possibility of hyper- or hyporesponsive neurons. By manipulating Ca(2+) signalling, an important process of most cells and thus also neurons, the function of neurons are inhibited making them hyper- or hyporesponsive (F. Haroon, 2012). To survive in the host neuronal cells, it is important for the parasite to block apoptosis of the cells. By blocking the apoptosis the parasite is interestingly capable of deactivating neurons but also their bystander cells (P. Vutova, 2007) (T. S. Lima, 2019). When the signalling of neurons is inhibited it may be the case that signalling of pathways inducing fear are cut off.

Next to the above-mentioned direct influences there are many indirect influences that possibly alter the behaviour of with *T. gondii* infected individuals. The alterations in behaviour are indirectly associated with neuroinflammation. Neuroinflammation is induced by, among others, peripheral mediators inducing neurodegeneration, neurotransmitters changes and alterations in neuronal morphology (A. Parlog D. S., 2015). As mentioned in the immunity against *T. gondii* many different peripheral mediators, inflammatory molecules, are activated. These inflammatory molecules are capable of initiating neuroinflammatory processes which influence the neuronal circuitry, eventually resulting in behavioural changes (E. Haroon, 2012). In *T. gondii* infection in mice there is a correlation seen between inflammation (factors) in the CNS and both reduced anxiety and increased exploration. However, the underlying mechanisms of the behavioural changes remain unknown (M. Boillat e. a., Neuroinflammation-Associated Aspecific Manipulation of Mouse Predator Fear by Toxoplasma gondii, 2020). Another possible factor in behavioural changes after *T. gondii* infection is neurodegeneration. This neurodegeneration can be caused by the inflammatory mediators which are potentially toxic for neurons (E. Zindler, 2010). The inflammatory factors, essentially neurotoxic mediators, are for example TNF-α and CCL2, factors involved in the immune system against *T. gondii*. Crossing the BBB these factors not only cause neuroinflammation which is mentioned before but can result in neurodegeneration (D. Kempuraj, 2016) (M. Lyman, 2014). Interestingly this may not be the case in *T. gondii* infection because the parasites capability to inhibit the neuronal degeneration (B. K. Jung, 2012). Inflammation has different influences on the brain, inflammatory factors can affect metabolism of multiple neurotransmitters such as serotonin, dopamine and glutamate. The synthesis, release and reuptake can be altered (A. H. Miller, 2013). Also, in *T. gondii* infection, causing inflammation, neurotransmitter levels became an interesting factor, however it is possible that there are differences between the genders in account. As mentioned in the direct factors effecting change in behaviour can be influenced by increased dopamine levels. This is not the only neurotransmitter associated with behavioural changes, also serotonin levels in the amygdala are altered (J. Gatkowska, 2013) (F. Ihara, 2016). In recent research there has been concluded that not only dopamine increases and serotonin decreases in *T. gondii* infected mice but that epinephrine and norepinephrine bitartrate are also altered (T. Wang, 2019). After *T. gondii* infection, alterations in the neurotransmitters are not always reported (H. H. Stibbs, 2016) (D. Goodwin, 2012). Activity of the neurotransmitters is critical in development and functioning of the physiological brain processes. So it is reasonable to assume that changes in neurotransmitter activity can be a factor in changes of behaviour (A. Parlog D. S., 2015). Not only molecules such as inflammatory factors or neurotransmitters and neurodegeneration are of influence in the change of behaviour in *T. gondii* infection but also changes in neuronal morphology. These changes in morphology can be retraction of dendrites (R. Mitra, 2013), morphometric changes (L. M. Zaniolo, 2012) and synaptic

deficits leading to changes in the morphology of neurons (A. Parlog e. a., 2014). The connection with behavioural changes and these different factors is made but again underlying mechanism seem to remain poorly understood.

T. gondii associated with human psychiatric diseases

Infection with *T. gondii* is associated with changed behaviour in rodents as explained earlier. However, *T. gondii* also seems to be associated with behavioural changes in human. Humans infected with the parasite seem to show impulsive aggression, specifically self-directed aggression (suicidal behaviour) (E. F. Coccaro, 2016). Not only (self-directed) aggression is detected in infected humans but also, especially in women, depression and anxiety problems are reported (J. Flegr, 2018). *T. gondii* is not only associated to these different behavioural changes in humans but also to different psychiatric disorders and symptoms. For examples connection between *T. gondii* and schizophrenia, Parkinson's and Alzheimer's diseases have been made (G. Syn, 2018) (K. S. Burgdorf, 2019). But also other disorders such as autism, antisocial personality disorder, obsessive compulsive disorder and Asperger syndrome have been associated with toxoplasmosis (J. Flegr, 2018).

Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease causing many cases of dementia. AD is associated with amyloid plaques and neurofibrillary tangles and leads to synaptic and neuronal loss (C. A. Schott, 2017). Involvement of *T. gondii* is still debated as for both involvement as non-involvement of the parasite in the cause of AD is found in research. In case control studies on the blood IgG and IGM against *Toxoplasma of* Alzheimer's patients and healthy volunteers similar results were found in both groups. Concentrations for anti-toxoplasma IgG are similar in non-patients and Alzheimer's patients. In both groups no positive results for IgM were found. The occurrence of toxoplasmosis in both groups were also similar. Insinuating that *T. gondii* is no risk for AD (M. Mahami-Oskouei, 2016). (C. E. Perry, 2016). However, all this does not indicate no involvement of *T. gondii* in AD. Animal models have been used to research the possible connection between *T. gondii* and AD. In different mice models with AD the possibility of involvement of *T. gondii* is reviewed. Results show that infection with *T. gondii* induces symptoms like those of AD, such as beta-amyloid (Aβ) immunoreactivity and hyperphosphorylated Tau. Infection with *T. gondii* induces neuroinflammation via cytokines and results in progression of AD in the brain. At the same time the brain is influenced by the *T. gondii* cyst present. All this shows the possibility that *T. gondii* can be involved in development of AD in individuals that already suffer from health problems (H. Mahmoudvand, 2016) (L. Torres, 2018).

Parkinson's disease

Parkinson's disease (DS) is a movement disorder caused by degeneration of the central nervous system. The most common symptoms are tremor, rigidity and bradykinesia (O. B. Tysnes, 2017). The association of *T. gondii* is also of interest in this mental disorder and is mainly assessed by case control studies. Results were like those in AD research, where Parkinson's and control volunteers have similar anti-toxoplasma IgM and IgG. However, interestingly it is still concluded that Parkinson's patients are more at risk for the infection of (S. Fallahi, 2017) (M. M. Oskouei, 2014). Another control case study from the same year again state that a significantly correlation between PD and *T. gondii* cannot be found. However, they did find the possibility of influence of *T. gondii* on the symptoms of PD (C. Alvarado-Esquivel, 2017).

Schizophrenia

Schizophrenia is seen in late teens and early twenties starting with subtle changes in behaviour such as social isolation, odd believes and hearing murmuring voices. This later manifests in hallucinations, delusion and

disorganized speaking (A. H. Ropper, 2019). In the cause of schizophrenia and their related psychiatric diseases the immune system is thought to be very much involved. This is supported by genetic, neuropathological and neuroimaging of different kinds of species. The brain can be influenced by inflammatory events resulting from defence against an infection. A great and well-studied example is toxoplasmosis (J. Xiao, 2018). *T. gondii* is capable of influencing host cell processes such as dopaminergic pathways and this also seems to be one of the underlying mechanisms involved in schizophrenia. Making a link between toxoplasmosis and the Schizophrenia (H. M. Elsheikha, 2016). In a case control study 2052 individuals are reviewed on *T. gondii* exposure, 1481 individuals have a psychiatric disorder and the 571 control individuals did not. Patients with schizophrenia and no recent onset of psychosis do not show the normal present increased chance for exposure (R.Yolken, 2017). High concentrations of antibodies against *T. gondii* are associated with lower levels of IgG3 and IgG4. Therefor schizophrenia patients infected with *T. gondii* can be more vulnerable for changes in immune response (N. Hamdani, 2018). Others have found similar percentage of *T. gondii* infection in Schizophrenia and control groups without any psychiatric disease. Indicating that the link between *L. gondii* and schizophrenia cannot be made in all studies. (L. Galli, 2019).

Depression and Suicidal behaviour

T. gondii is associated with many different psychiatric diseases as mentioned earlier. It is also linked to depression and possible suicidal behaviours. In specific it is linked to depression in pregnant women. Screening depressed pregnant women and controls on anti-toxoplasma IgG give no significant difference between both groups. These results do not support the suspected link between *T. gondii* and prenatal depression. Interestingly the IgG titer in depressed woman was significantly higher than in the control group. A positive correlation is found between this high titer and the scores on the Edinburgh Postpartum Depression Scale (EDPS). The mean EDPS score was higher in the *T. gondii* positive depressed women (M. N. Shiadeh, 2016). (C. Alvarado-Esquivel, 2017). Depression in children and adolescents is also linked to *T. gondii* infection. Comparing a group of children and adolescents diagnosed with depression with children and adolescent without depression, significantly more depressed individuals were seropositive for *T. gondii.* The seropositivity in patients showing self-directed aggression was also significantly higher than in patients who did not show this kind of behaviour. Indicating there is a possible link between *T. gondii* infection and self-directed aggression (S. Y. Sapmaz, 2019), however the statement of correlation between that infection and self-directed aggression and suicidal behaviour is not completely certain (A. L. Sutterland, 2019). *T. gondii* probably does not directly lead to the suicidal behaviour but it does influence the psychiatric problems that are encountered in suicidal individuals. In a group of suicide attempters and control individuals again the group of suicide attempters shows more seropositive individuals. (J. Bak, 2018).

Schizophrenia patients often show (nonfatal) suicidal self-directed violence, as mentioned *T. gondii* has already been associated with schizophrenia but it might also effect the self-directed violence seen in this disease (M. Ansari-Lari, 2017). Another factor in suicidal behaviour in schizophrenia patients is high blood kynurenine. However there is stated that it is probably a cumulative effect of risks factors such as the parasite, kynurenine, etc. (O. Okusaga, 2016).

Discussion

T. gondii is one of the most common parasites in human infections but it does not only infect humans but all warm-blooded animals, birds and reptiles. Felids are the definite host making cats of great importance in *T. gondii* transmission (J. L. Jones, 2018) (D. L. Zulpo, 2018). As the parasites infects many different

species, rodents do not stay unharmed. Interestingly it seems this intermediate host is stripped of the fear for predators such as the definite host, cats (K. Servick, 2020) Development of this fatal attraction is stated to be caused by lowering of anxiety and increasing explorative behaviour by *T. gondii* (M. Boillat e. a., 2020). This gave rise to many different questions but mainly: how does this parasite influence the brain and its hosts behaviour?

Starting with the focus on the life cycle of *T. gondii* it has become very clear that *T. gondii* is a very complicated organism. Using 3 different kinds of cysts for survival tachyzoites forming groups, bradyzoites in tissues and sporozoites. The sporozoites most important in the transmission starting infection in many hosts such as de rodents (J. P. Dubey, 2002). When infected with any pathogen an immune response is activated which possibly can be used in the advantages of *T. gondii*. Immune response activates many different cytokines, chemokines and immune cells as a defence against the parasite. *T. gondii* however is capable to use this in its advantage by invading immune cells to get easy access to many different organs and even cross the BBB (A. Krishnan, 2016). This makes clear that *T. Gondii* has a very defined system to gain excess to not only different hosts but also to their brain.

When entered the brain the parasite seems to have a great influence as different changes in behaviour are witnessed (M. Berenreiterova, 2011). Trying to understand why *T. gondii* induces behavioural changes, first guess was a possible prevalence for a specific brain area where the parasite is located. However, it is not the case that there is a specific prevalence for a brain area but that the location of *T. gondii* cysts is specific for different kinds of behavioural changes. Cysts in motor cortices lead to motor and coordination deficits. Cysts in visual cortex and olfactory bulb lead to sensory deficits. Cysts in the dorsal hippocampus leads to cognitive deficits. And cysts in the amygdala and ventral hippocampus lead to decreased aversion to predators (M. Berenreiterova, 2011). This makes the amygdala the most interesting region of the infected brain in the case of behavioural changes towards predators. However, it is difficult to say why and how exactly the parasite is manifested in this brain area. This because research shows different results regarding a possible prevalence for specific brain areas and the possible reason behind it (P. K. House, 2011) (F. Ihara, 2016) (D. Goodwin, 2012). Still further research on the connection between the amygdala and the behavioural changes towards predators can be of great use. This because of the involvement of this brain area in the detection of threat (C. Mendez-Bertolo, 2016). And the importance to understand the mechanism underlying the changes in the amygdala where the behavioural originates from.

Influence on the brain (among others on the amygdala) mostly seems to be in the form of changes in neuronal function and structure in a direct and indirect manner (A. Parlog D. S., 2015). *T. gondii* contains two different genes coding for L-DOPA (precursor of dopamine), resulting in higher dopamine concentrations in *T. gondii* infected individuals (E. A. Gaskell, 2009) (F. Ihara, 2016) (E. Prandovsky, 2011). The increased levels of dopamine possibly reduce the fear experienced by infected individuals. However it is not clear what the exact underlying mechanism of reducing of fear is (F. Ihara, 2016) (E. Prandovsky, 2011). Direct influence on neuronal functioning is manifested by either hyperresponsiveness, hyporesponsiveness or blocking of apoptosis in neurons. This alters communication between infected and non-infected neurons and can even result in inactivation of both (F. Haroon, 2012) (P. Vutova, 2007) (T. S. Lima, 2019). Next to the direct influences on neuronal functioning by *T. gondii* there are also many indirect factors causing neuroinflammation which is of influence on neurons. *T. gondii* activates inflammatory factors which are linked to the reduced anxiety and increased exploration. However, the underlying

mechanisms of the behavioural changes remain unknown (M. Boillat e. a., Neuroinflammation-Associated Aspecific Manipulation of Mouse Predator Fear by Toxoplasma gondii, 2020). Inflammatory factors act as neurotoxic mediators causing neurodegeneration (D. Kempuraj, 2016). Interestingly this may not be the case in *T. gondii* infection because the parasites capability to inhibit the neuronal degeneration (B. K. Jung, 2012). Inflammatory factors can also affect metabolism of multiple neurotransmitters such as dopamine and serotonin (A. H. Miller, 2013). Also changes in morphology can be of influence in behavioural changes such as retraction of dendrites (R. Mitra, 2013), morphometric changes (L. M. Zaniolo, 2012) and synaptic deficits leading to changes in the morphology of neurons (A. Parlog e. a., 2014).

Current situation in research is that many factors have been found in connection to behavioural changes, as they are mentioned above. Direct influences on the functioning of neurons, which can be hyperresponsiveness, hyporesponsiveness or are inactive which can alter the reaction of the brain on stress or threat signs. As these signals cannot be interpreted in the right way it is reasonable that this results in the loss of fear even when encountered with threat signs such as predators. This is also the case with the indirect factors altering functioning and mainly signalling of neurons. Where concentrations of neurotransmitters are altered, development and functioning of the physiological brain processes are also altered. Activity of the neurotransmitters is critical in development and functioning of the physiological brain processes. So, it is reasonable to assume that changes in neurotransmitter activity can be a factor in changes of behaviour. Changes in the morphology of neurons cause changes in signalling between the neurons. Again, indicating the inability to read, or misinterpretation of, threat like signs of predators causing the fatal attraction or loss of fear seen in the *T. gondii* infected rodents. There are so many factors that have a possible share in the cause of the fatal attraction in *T. gondii* infected rodents but most of the underlying mechanisms and possible links between the factors are poorly understood. This makes it a high priority to further investigate this phenomenon so that mechanisms behind different factors are better understood and possibly can be used in medical research for human mental illness.

If infection in rodents can alter the brain and its function, why could this not be the case in humans. It seems that toxoplasmosis in humans indeed is linked to many different psychiatric diseases such as schizophrenia, Parkinson's disease and Alzheimer's disease (G. Syn, 2018) (K. S. Burgdorf, 2019) but also diseases such as self-directed aggression, depression, autism and Asperger syndrome (J. Flegr, 2018). Even though multiple psychiatric diseases have been linked to *T. gondii,* it is hard to significantly link *T. gondii* to psychiatric diseases in case studies. It does seem to be the case that *T. gondii* does not influence the diseases and in specific depression directly but it can influence the risk and severity of the symptoms that the patients experience (J. Bak, 2018) (M. N. Shiadeh, 2016). This again shows the importance to fully understand underlying mechanisms of *T. gondii* and how it is capable of altering brain function and behaviour before hard conclusions can be made about how the parasite changes behaviour, how it is connected to human psychiatric diseases and eventually how it can be used in medical research for possible treatment.

Afterword

I would like to use these last words of the thesis to thank my thesis mentor Robbert Havekes for giving the opportunity for and the help in writing this thesis. I have not only learned a lot about the *T. gondii* infection, and it's influences on behaviour. I also learned a lot about doing literature research, reading and writing on an academic level.

References

A. H. Miller, e. a. (2013, March 6). Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depression and Anxiety, 30*(4).

A. H. Ropper, S. R. (2019, October 31). Schizophrenia. *New England Journal of Medicine, 381*(18), 1753-1761.

A. Krishnan, D. S.-F. (2016, February 24). Breaching the wall for brain access. *Nature Microbiology, 1*.

A. L. Sutterland, e. a. (2019, March 26). Driving us mad: the association of Toxoplasma gondii with suicide attempts and traffic accidents – a systematic review and meta-analysis. *Psychological Medicine, 49*, 1608-1623.

A. M. Tenter, A. R. (2000, November). Toxoplasma gondii: from animals to humans. *International Journal for Parasitology, 30*(12-13), 1217-1258.

A. Parlog, D. S. (2015, March). Toxoplasma gondii-induced neuronal alterations. *Parasite Immunology, 37*(3), 159-170.

A. Parlog, e. a. (2014). Chronic murine toxoplasmosis is defined by subtle changes in neuronal connectivity. *Disease Models and Mechanisms, 7*, 459-469.

A. Vyas, e. a. (2007, April 10). Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. *PNAS, 104*(15), 6442-6447.

B. K. Jung, e. a. (2012, March). Toxoplasma gondii infection in the brain inhibits neuronal degeneration and learning and memory impairments in a murine model of Alzheimer's disease. *PloS One, 7*(3), 1-11.

C. A. Scanga, e. a. (2002, June 15). Cutting Edge: MyD88 Is Required for Resistance to Toxoplasma gondii Infection and Regulates Parasite-Induced IL-12 Production by Dendritic Cells. *The journal of Immunology, 168*(12), 5997-6001.

C. A. Schott, J. L. (2017, September 4). Alzheimer's disease. *European Journal of Neurology, 25*(1).

C. Alvarado-Esquivel, e. a. (2017, March 6). Lack of association between Toxoplasma gondii exposure and depression in pregnant women: a case-control study. *BMC Infectious Diseases, 17*(190).

C. Alvarado-Esquivel, e. a. (2017, February 13). Toxoplasma gondii exposure and Parkinson's disease: a case–control study. *Infectious diseases, 7*(2).

C. E. Perry, e. a. (2016, April-June). Toxoplasmosis and Alzheimer: can Toxoplasma gondii really be introduced as a risk factor in etiology of Alzheimer? *Alzheimer Disease & Associated Disorders, 30*(2), 123-126.

C. Mendez-Bertolo, e. a. (2016, June 13). A fast pathway for fear in human amygdala. *Nature Neuroscience, 19*, 1041-1049.

D. Degrandi, e. a. (2013, January 2). Murine Guanylate Binding Protein 2 (mGBP2) controls Toxoplasma gondii replication. *PNAS, 110*(1), 294-299.

D. Goodwin, e. a. (2012, August). CONGENITAL INFECTION OF MICE WITH TOXOPLASMA GONDII INDUCES MINIMAL CHANGE IN BEHAVIOR AND NO CHANGE IN NEUROTRANSMITTER CONCENTRATIONS. *The Journal of Parasitology, 98*(4), 706-712.

D. Kempuraj, e. a. (2016, November 18). Neuroinflammation Induces Neurodegeneration. *Journal of Neurosurgery: Spine, 1*(1), 1003.

D. L. Zulpo, e. a. (2018, January 15). Toxoplasma gondii: A study of oocyst re-shedding in domestic cats. *Veterinary Parasitology, 249*, 17-20.

E. A. Gaskell, J. S. (2009, March 11). A unique dual activity amino acid hydroxylase in Toxoplasma gondii. *PloS One, 4*(3).

E. F. Coccaro, e. a. (2016, March). Toxoplasma gondii Infection: Relationship With Aggression in Psychiatric Subjects. *The journal of clinical psychiatry, 77*(3), 334-341.

E. Gilot-Fromont, e. a. (2012). The life cycle of Toxoplasma gondii in the natural environment. In *Toxoplasmosis: Recent Advances* (pp. 3-23).

E. Haroon, C. L. (2012). Psychoneuroimmunology Meets Neuropsychopharmacology: Translational Implications of the Impact of Inflammation on Behavior. *Neuropsychopharmacology volume, 37*, 137-162.

E. M. Selleck, e. a. (2013, January). Guanylate-binding Protein 1 (Gbp1) Contributes to Cell-autonomous Immunity against Toxoplasma gondii. *PLoS Pathogens, 9*(1), 1-15.

E. Prandovsky, e. a. (2011, September 21). The Neurotropic Parasite Toxoplasma Gondii Increases Dopamine Metabolism. *PloS One, 6*(9).

E. Zindler, F. Z. (2010, December). Neuronal injury in chronic CNS inflammation. *Best Practice & Research Clinical Anaesthesiology*(24), 551-562.

F. Haroon, e. a. (2012, April 18). Toxoplasma gondii Actively Inhibits Neuronal Function. *PloS one, 7*(4).

F. Ihara, e. a. (2016, September 19). Toxoplasma gondii Infection in Mice Impairs Long-Term Fear Memory Consolidation through Dysfunction of the Cortex and Amygdala. *ASM Infection and Immunity, 84*(10), 2861-2870.

G. Syn, e. a. (2018, November). Epigenetic dysregulation of host gene expression in Toxoplasma infection with specific reference to dopamine and amyloid pathways. *Infection, Genetics and Evolution, 65*, 159-162.

H. H. Stibbs, e. a. (2016, November 15). Changes in brain concentrations of catecholamines and indoleamines in Toxoplasma gondii infected mice. *Annals of Tropical Medicine & Parasitology, 79*(2), 153-157.

H. M. Elsheikha, D. B. (2016). The known and missing links between Toxoplasma gondii and schizophrenia. *Metabolic Brain Disease, 31*, 749-759.

H. Mahmoudvand, e. a. (2016, December). TOXOPLASMA GONDII INFECTION POTENTIATES COGNITIVE IMPAIRMENTS OF ALZHEIMER'S DISEASE IN THE BALB/C MICE. *The Journal of Parasitology, 102*(6), 629-635.

J. A. Kochanowsky, A. A. (2018, July 23). Toxoplasma gondii. *CellPress, Current Biology, 28*(14), 770-771.

J. Alberti, e. a. (2000, July). CCR5 provides a signal for microbial induced production of IL-12 by CD8α+ dendritic cells. *Nature immunology, 1*, 83-87.

J. Bak, e. a. (2018, Februari 28). The Association between Suicide Attempts and Toxoplasma gondii Infection. *Clinical Psychopharmacology and Neuroscience, 16*(1), 95-102.

J. Flegr, J. H. (2018, July). Toxoplasmosis, but not borreliosis, is associated with psychiatric disorders and symptoms. *Schizophrenia research, 197*, 603-604.

J. G. Montoya, O. L. (2004, June 12). Toxoplasmosis. *The Lancet, 363*(9425), 1965-1976.

J. Gatkowska, e. a. (2013, January). Sex-dependent neurotransmitter level changes in brains of Toxoplasma gondii infected mice. *Experimental Parasitology, 133*(1), 1-7.

J. L. Jones, e. a. (2018). Toxoplasma gondii Infection in the United States, 2011–2014. *The American Journal of Tropical Medicine and Hygiene, 96*(2), 551-557.

J. P. Dubey, e. a. (2002, August). Tachyzoite-induced life cycle of Taxoplasma gondii in cats. *The Journal of Parasitology, 88*(4), 713-717.

J. P. Dubey, e. a. (2002, August). Tachyzoite-Induced Life Cycle of Toxoplasma gondii in Cats. *The Journal of Parasirology, 88*(4), 713-717.

J. P. Hunn, e. a. (2008, September 4). Regulatory interactions between IRG resistance GTPases in the cellular response to Toxoplasma gondii. *The EMBO Journal, 27*, 2495-2509.

J. Xiao, e. a. (2018, June 8). Toxoplasma gondii: Biological Parameters of the Connection to Schizophrenia. *Schrizophrenia Bulletin, 44*(5), 983-992.

K. S. Burgdorf, e. a. (2019, July). Large-scale study of Toxoplasma and Cytomegalovirus shows an association between infection and serious psychiatric disorders. *Brain, Behavior and Immunty, 79*, 152-158.

K. Servick, e. a. (2020, Januari 14). Brain parasite may strip away rodents' fear of predators—not just of cats. *Science, News*.

L. Galli, e. a. (2019, January 21). Lack of circulating toxoplasma gondii DNA in seropositive patients with bipolar or schizophrenia spectrum disorders. *Psychiatry Research, 273*, 706-711.

L. Kasper, e. a. (2004, March 9). Toxoplasma gondii and mucosal immunity. *International Journal for Parasitology, 34*(3), 401-409.

L. M. Zaniolo, e. a. (2012, February). Toxoplasma gondii infection causes morphological changes in caecal myenteric neurons. *Experimental Parasitology, 130*(2), 103-109.

L. Torres, e. a. (2018, Februari 23). Toxoplasma gondii alters NMDAR signaling and induces signs of Alzheimer's disease in wild-type, C57BL/6 mice. *Journal of Neuroinflammation, 15*(1).

M. Ansari-Lari, H. F. (2017, July 22). Association of Toxoplasma gondii infection with schizophrenia and its relationship with suicide attempts in these patients. *Tropical Medicine & International Health, 22*(10).

M. Berdoy, J. W. (2000, August 7). Fatal attraction in rats infected with Toxoplasma gondii. *Proceeedings: Biological Sciences, 267*(1452), 1591-1594.

M. Berenreiterova, e. a. (2011). The Distribution of Toxoplasma gondii Cysts in the Brain of a Mouse with Latent Toxoplasmosis: Implications for the Behavioral Manipulation Hypothesis. *PLoS ONE, 6*(12).

M. Boillat, e. a. (2020, January 14). Neuroinflammation-Associated Aspecific Manipulation of Mouse Predator Fear by Toxoplasma gondii. *Cell Reports, 30*(2), 20-334.

M. Boillat, e. a. (2020, January 12). Neuroinflammation-Associated Aspecific Manipulation of Mouse

Predator Fear by Toxoplasma gondii. *Cell Reports, 30*(2), 320-334.

M. Boillat, e. a. (2020, January 14). Predator Cat Odors Activate Sexual Arousal Pathways in Brains of Toxoplasma gondii Infected Rats. *Cell Reports, 30*(2), 320-324.

M. I. Sasai, A. M. (2018, February 2). Host immune responses to Toxoplasma gondii. *International Immunology, 30*(3), 113-119.

M. Lyman, e. a. (2014). Neuroinflammation: The role and consequences. *Neuroscience Research, 79*, 1-12.

M. M. Oskouei, e. a. (2014, October 14). The correlation between Toxoplasma gondii infection and Parkinson's disease: a case-control study. *Journal of Parasitic Diseases, 40*, 872-876.

M. Mahami-Oskouei, e. a. (2016). Toxoplasmosis and Alzheimer: can Toxoplasma gondii really be introduced as a risk factor in etiology of Alzheimer? *Parasitology Research, 115*, 3169-3174.

M. N. Shiadeh, e. a. (2016, August 08). The correlation between Toxoplasma gondii infection and prenatal depression in pregnant women. *European Journal of Clinical Microbiology & infectious diseases, 35*, 1829-1835.

M. Yamamoto, e. a. (2012, August 24). A Cluster of Interferon-γ-Inducible p65 GTPases Plays a Critical Role in Host Defense against Toxoplasma gondii. *CellPress Immunity, 37*(2), 302-313.

N. Hamdani, e. a. (2018, July 27). Immunoglobulin sub-class distribution in bipolar disorder and schizophrenia: potential relationship with latent Toxoplasma Gondii infection. *BMC Psychiatry, 18*(1), 239.

O. B. Tysnes, A. S. (2017, February 1). Epidemiology of Parkinson's disease. *Journal of Neural Transmission, 124*, 901-905.

O. Okusaga, e. a. (2016, January). Combined Toxoplasma gondii seropositivity and high blood kynurenine – Linked with nonfatal suicidal self-directed violence in patients with schizophrenia. *Journal of Psychiatric Research, 72*, 74-81.

P. K. House, A. V. (2011). Predator Cat Odors Activate Sexual Arousal Pathways in Brains of Toxoplasma gondii Infected Rats. *PLoS ONE, 6*(8), 1-4.

P. Vutova, e. a. (2007). Toxoplasma gondii inhibits Fas/CD95-triggered cell death by inducing aberrant processing and degradation of caspase 8. *Cell Microbiology, 9*, 1556-1570.

R. Mitra, R. M. (2013). Toxoplasma gondii infection induces dendritic retractionin basolateral amygdala accompanied by reducedcorticosterone secretion. *Disease Models & Mechanism, 6*, 516-520.

R.Yolken, E. F. (2017, November 6). Evidence of increased exposure to Toxoplasma gondii in individuals with recent onset psychosis but not with established schizophrenia. *PLOS Neglected Tropical DIseases, 11*(11), 1-10.

S. Berger, e. a. (2019). Toxoplasmosis. Gideon Informatics, Inc.,.

S. Fallahi, e. a. (2017, September). Parkinson's disease and Toxoplasma gondii infection: Sero-molecular assess the possible link among patients. *Acta Tropica, 173*, 97-101.

S. J. Hadfield, E. C. (2017, December). Toxoplasmosis. *Medicine, 45*(12), 763-766.

S. K. Bliss, B. A. (2000, October 15). Rapid Recruitment of Neutrophils Containing Prestored IL-12 During Microbial Infection. *The journal of Immunology, 165*(8), 4515-4520.

S. Tyebji, e. a. (2019, January). Toxoplasmosis: A pathway to neuropsychiatric disorders. *Neuroscience & Biobehavioral Reviews, 96*, 72-92.

S. Y. Sapmaz, e. a. (2019, August). Relationship between Toxoplasma gondii seropositivity and depression in children and adolescents. *Psychiatric Research, 278*, 263-267.

T. S. Lima, M. B. (2019, April 16). Mechanisms of Human Innate Immune Evasion by Toxoplasma gondii. *Frontiers in Cellular and Infection Microbiology, 9*(103).

T. Wang, e. a. (2019, February 1). From inflammatory reactions to neurotransmitter changes: Implications for understanding the neurobehavioral changes in mice chronically infected with Toxoplasma gondii. *Behavioural Brain Research, 359*, 737-748.

V. B. Carruthers, e. a. (2002, February). Host cell invasion by the opportunistic pathogen Toxoplasma gondii. *Acta Tropica, 81*(2), 111-122.