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Noradrenergic Signaling- Neuroinflammation Cross-talk Regulates *Toxoplasma gondii*-Induced Behavioral Changes

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Abstract

Infections of the nervous system elicit neuroimmune responses and alter neurotransmission, affecting host neurological functions. Chronic infection with the *Toxoplasma gondii* parasite correlates with neurological disorders in humans and alters behavior in rodents. Here, we propose that the crosstalk between neurotransmission and neuroinflammation may underlie these cognitive changes. We discuss how *T. gondii* infection suppresses noradrenergic signaling and how the restoration of this pathway improves behavioral aberrations, suggesting that altered neurotransmission and neuroimmune responses may act in concert to perturb behavior. This interaction might apply to other infectious agents, such as viruses, that elicit cognitive changes. We hypothesize that neurotransmitter signaling in immune cells can contribute to behavioral changes associated with brain infection, offering opportunities for potential therapeutic targeting.

***Toxoplasma gondii* modifies host behavior**

Recent work has found that microbes, including the intestinal microbiota and viral infections, can influence brain function and behavior [1]. While microbial effectors may exert a direct effect on neurons and dysregulate neurotransmitter production, immune responses against microbes might also influence neuronal activity. In recent years, strong evidence has indicated that 'immune' soluble mediators (e.g., cytokines), produced by a variety of immune cells at steady-state or during neuroinflammation, have a profound impact on brain physiology [2]. Indeed, neuroinflammation is thought to play an important role in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) [3]. Conversely, neurotransmitters or neuroendocrine mediators constitute key modulators of immune cells [4], highlighting the potency and complexity of neuroimmune networks.

The brain-persisting, zoonotic, parasite *Toxoplasma gondii* is one of the best-studied examples of a microbe modifying host behavior. Despite the induction of robust cellular immune responses, this intracellular protozoan persists in host cells of the retina, muscles, and central nervous system (CNS) in the form of **bradyzoites** – contained within intra-neuronal cysts and constituting the latent stages of *T. gondii* infection (**Figure 1 and Box 1**). Cognitive alterations induced by *T. gondii* occur during chronic stages of infection [4]. In rodents, infection causes hyperlocomotion, reduced anxiety, decreased **neophobia**, and predator vigilance [5]. A striking consequence of chronic *T. gondii* infection in rodents is the loss and reversal of innate aversion toward predator scents, such as the loss of fear of feline urine [6-8]. However, a recent article challenged the specificity towards a feline predator scent [9]. Since the sexual reproduction stages of *T. gondii* can only occur in feline intestines, this intriguing phenotype is thought to represent **host manipulation**, increasing the likelihood that parasite transmission occurs in its definitive host [10-14]. In addition, *T. gondii* infection in rodents also affects learning, and impairs spatial working memory and fear memory consolidation [12-14].

In humans, studies have relied on serum antibody seropositivity against *T. gondii* antigens.to infer the presence of parasite cysts in the CNS ; indeed, this is necessary as direct detection of cysts in the brain is not feasible (other than post-mortem)**.** *T. gondii* infection has been associated neuropsychiatric disorders and this may be more of a concern than previously believed [15]. In the general population, one of the most striking correlations of infection with neurological disease has been with schizophrenia. Specifically, a recent prospective study of a large cohort of Danish individuals provided the first hint of causality between *T. gondii* infection and schizophrenia in humans. Seropositive individuals presented increased odds of being diagnosed with schizophrenia disorders, and additionally, this association was stronger if temporality was accounted for – namely, when considering cases in which seropositivity preceded the diagnosis of schizophrenia [16].

Although the mechanism(s) responsible for these behavioral changes are complex and the details remain ill-defined, it is clear that *T. gondii* infection down-regulates concentrations of

the catecholamine norepinephrine (NE, also called noradrenaline) in the cortex, amygdala, and locus coeruleus of rodents; the NE levels were correlated with noradrenergic signaling-linked behaviors (ie. arousal, hyperactivity, sociability) in infected mice [6, 13]. Beyond its neuron-intrinsic role in neurotransmission, noradrenergic signaling negatively modulates the function of many immune cells. Experiments performed *in vitro* with agonists/antagonists or *in vivo* in mice with conditional deletion of noradrenergic receptors show suppressive effects on the production of pro-inflammatory cytokines by brain-resident cells (microglia, astrocytes) [17] as well as peripheral lymphocytes and myeloid cells [18, 19]. In this opinion article, we argue that behavioral perturbations caused by *T. gondii* infection are induced by the drop in NE concentrations, unleashing pro-inflammatory activities in immune cells, and, in turn, elevating neuroinflammation.

Intra-neuronal *T. gondii* alters neurotransmitters

Several changes in neurotransmission accompany chronic *T. gondii* infection that can influence behavior. First, catecholamines are altered. Excessive dopamine amounts have been visualized in infected mouse neurons via immunofluorescence, as well as increased dopamine and dopamine metabolites in infected rodent brains, although there is disparity between published studies [13, 20, 21]. These differences might be due to the diversity of the parasite and mouse strains used, and the lack of standardized experimental conditions. Synthesis of the catecholamine NE (**Box 2**), was decreased more than 30-fold in the brains of *T. gondii*-infected animals relative to uninfected Lister Hooded rats [6, 22]. As this represents a decrease in the enzyme that produces NE from dopamine, the decreased dopamine metabolism may, at least partially, explain the observed increase in dopamine. Functionally, dopamine's potential involvement in behavioral alterations is supported by the observation that anti-psychotics that bind dopamine receptors, dopamine D2 antagonist haloperidol and dopamine selective uptake inhibitor GBR 12909, have blocked infection-induced loss of cat urine fear in rats [23] and holeboard test for anxiety and exploratory activity in mice [24], respectively. Furthermore, infection activates neural activity, measured as c-fos concentration, in the dopamine-responsive rat medial amygdala, a brain region that is involved in fear and stress [25]. Second, elevated extracellular glutamate(another classic neurotransmitter) and reduced concentrations of glutamate transporter Glt-1 have been observed in both *T. gondii*-infected encephalitis-susceptible C57Bl/6 and encephalitis-resistant BALB/cmice [26]. Pharmacological enhancement of the transporter with the β-lactam antibiotic ceftriaxone re-established normal glutamate concentrations in the brain's extracellular space but did not rescue the loss of **risk-aversive behavior**, suggesting that astrocytic glutamate dysregulation was not crucial to this behavioral modification**.**

Third, tissue cyst infection of C57BL/6 mice with type II ME49 *T. gondii* subtly disrupted the distribution (but not the amount) of the principal enzyme involved in γ-aminobutyric acid (GABA) synthesis in the hippocampus based on protein and RNA analysis and

immunofluorescence –an essential neurotransmitter for inhibitory synapses that prevents the onset of seizures [27]. Extending this experiment, it was recently found that activated microglia displace or phagocytose synaptic elements of GABA inhibitory nerve terminals [28]. Fourth, expression of the neuropeptide arginine vasopressin, based on RT-PCR, was increased in the amygdala of *T. gondii*-infected rats [29].

Despite all these observations, the mechanism(s) responsible for behavior and neurotransmission changes remain(s) unclear. Given their location inside neurons, *T. gondii* cysts are ideally positioned to manipulate neurotransmitters. Yet only a small percentage of the neurons contain cysts, which cannot explain the observed global changes in neurotransmitters and the consistent behavior effects on rodents. Thus, experimentation does not support the hypothesis that selective tropism of *T. gondii* for particular brain regions underlies cognitive alterations [8, 9, 25, 30].

Nevertheless, multiple studies in rats and mice show that *T. gondii* infection leads to degradation of synaptic structure and **dendritic arbor**; in addition, reports indicated that maintaining synaptic structural and functional integrity (synaptophysin and PSD95) were diminished in mice, altering neuronal integrity and connectivity that could, in turn, produce irregular neurotransmission [26, 31, 32]. These findings suggest that infection of neurons with cysts may specifically alter their interactions which could result in behavior changes. Nevertheless, synaptic structure changes have not been restricted to infected neurons and this type of degradation might be the result of destructive autoimmune responses to infection as in autoimmune encephalitis; areas that will need to be further elucidated [33].

While changes in neurotransmission certainly underlie cognitive perturbations, factors involved in neuroinflammation and crosstalk between immune responses and neurotransmitters will also need to be considered when aiming to elucidate the putative regulatory mechanisms of neurotransmission and behavior during chronic *T. gondii* infection, as discussed below.

Chronic *T. gondii* infection elicits neuroimmune responses

To consider how neurotransmission may interact with neuroinflammation, the immune response to chronic infection needs to be understood. Beyond the prominent type 1 immune responses that are mobilized in response to *T. gondii* infection in the periphery (**Box 1**) , local immune reactions develop within the infected CNS, involving both peripherally-recruited and resident neuroimmune cells [34]. Although *T. gondii* infection is controlled in rats leading to chronic, latent infection, in mice, infection can lead to either a controlled latent infection (e.g. BALB/c infected with **type II parasites**) or a chronic progressive encephalitis (e.g. C57BL/6 infected with **type I parasites**) depending on the combination of parasite and mouse strains.

To date, the key determinant regulating the clinical status (encephalitis *vs* latency) is the presence of robust infiltrating $CD8^+$ T cells in mice ,targeting an antigen from *T. gondii* that is efficiently presented by MHC class I molecules on infected cells [34-36]. MHC I presentation

of parasite antigens by infected neurons plays a pivotal role in hampering cyst development in a C57BL/6-based model of *T. gondii* latency; this process occurs regardless of whether the protective antigen is expressed by the bradyzoite stages in a model with recombinant parasites expressing a tractable CD8 T cell model antigen, suggesting that immune surveillance of latent infection in the CNS might be achieved via $CD8^+$ T cell recognition of **tachyzoite**-infected neurons [37]. Beyond IFN-γ production, which is essential for the upregulation of MHC I presentation by infected mouse neurons [37], T cell-mediated protection mechanisms likely involve perforin-dependent cytotoxicity by $CD8^+$ T cells from studies of *T. gondii* in perforin knockout mice [38].

The resident neuroimmune cells, microglia and astrocytes, generate a frontline response to CNS infection. Microglia are activated close to *T. gondii* replication foci, and they represent more than 10% of parasite-exposed cells in the CNS, based on studies with Cre recombinase expressing *T. gondii* in mice that express GFP in a Cre-dependent fashion [39]. Throughout infection, microglia release multiple chemokines and cytokines; including IL-1β, TNF-α [40], and IFN-γ [41]. Concomitantly, anti-inflammatory pathways, such as TGF-β secretion and the inhibition of nitric oxide production [42], occur in mice microglia. Astrocytes contribute to the brain's inflammatory milieu through the production of MCP-1 and IL-1α [43], and TGF-β [44] in CBA/J mice. Moreover, astrocytes regulate *T. gondii* infection via IFN-γ as mice in which astrocytes are unable to signal through STAT1 display higher brain parasite loads with changes in activation status of T cells, macrophages, and microglia [45].

Also, during *T. gondii* infection, other peripheral innate immune cells infiltrate the brain, although their respective roles remain ill-defined. $CD11c^+$ cells, which include dendritic cells, form **granuloma** structures that contain tachyzoites and interact with parasite-specific $CD8^+$ T cells [46]. Upon brain recruitment, $CCR2^+$ $Ly6c^{high}$ monocytes produce iNOS, pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, TNF), as well as the regulatory cytokine IL-10 and since depletion of this monocyte subset elevated parasite load and decreased survival of infected mice, these are involved in the control of *T. gondii* during chronic infection [47].

Thus, the strong neuroimmune responses that develop and persist throughout chronic *T. gondii* infection can alter neurotransmitter signaling, and we speculate that these in turn, might contribute to influencing behavioral changes, at least in rodents.

Effects of neuroinflammation on neural functioning

Strong emerging evidence from mouse models shows that local neuroimmune cross-talk impacts cerebral functions in several ways. First, cytokines negatively or positively influence cognition. For instance, using knock-out mice and a panel of behavioral tests, IFN-γ was shown to promote GABAergic signaling, which positively regulates social interactions and mood [48]. Conversely, still using genetically modified mouse models as well as IL-1 receptor antagonist treatment, it was reported that the inflammatory cytokine IL-1β is implicated in driving behavioral abnormalities and cognitive decline [49, 50]. Second, synaptic stripping by

activated microglia can lead to cognitive sequelae following mouse exposure and recovery from neurotropic viral infections caused by lymphocytic choriomeningitis virus, Zika virus and West Nile virus [51, 52]. Lastly, the presence of intra-cerebral $CD8^+$ T cells was suggested to perturb brain homeostasis in aging mice. The infiltration of $CD8^+$ T cells in old neurogenic niches negatively impacts neural stem cell proliferation, which may contribute to age-related decline [53].

In conclusion, neuroinflammatory cues can substantially impact behaviors. Neuroinflammation has also been associated with cognitive decline during AD and PD [3]. For *T. gondii*, the evidence linking neuroinflammation and behavior is scarce. With the necessity of continuous immunity to maintain the parasite in its latent form, it is tempting to assume that the association with the behavior changes is causal [9]. Boilatt et al found that the time spent by infected mice in their home compartment in a predator urine aversion test correlated with serum levels of proinflammatory IFN-γ, TNF, and IL-12b and anti-inflammatory IL-27 and Alox5ap [9]. IFN-γ also correlated with reduced anxiety and increased exploration in the infected mice behavior [9]. Future experiments need more robust demonstrations of the link between neuroinflammation with behavior.

The noradrenergic system as a master regulator of behavior and neuroinflammation

The noradrenergic system, centered in the *locus coeruleus* in the hindbrain, extends efferents (dendrites extending from the neuron cell body for delivery of neurotransmitter) to most brain regions and is a key modulator of neuronal activity through NE release [54]. NE is released continuously, and the *locus coeruleus* is activated in response to stress or infection [55, 56]. By signaling through adrenergic receptors (AR) (**Box 2**), NE is a master regulator of multiple cognitive functions. NE activates neurons invoking fear memory, increased alertness, arousal, locomotor activity, and learning and memory; all traits which are dysregulated in *T. gondii*-infected animals (discussed above) [55, 56]. NE from the activated *locus coeruleus* in animals is also released into the body and can act systemically [55, 56].

As well as its role in neuronal signaling, NE is involved in regulating inflammation. NE signaling in the CNS-resident immune cells, astrocytes and microglia, suppress the production of various pro-inflammatory factors, such as iNOS, IL-1β, IL-6, and TNF-α [55, 56], in part by inhibiting NF-κB transcriptional activity [17]. Astrocytes and microglia also possess ARs with high affinity for NE. As a result of NE signaling, microglia are activated for neuronal network surveillance and synaptic maintenance [57, 58] (**Figure 2**). In the brain, AR signaling also exerts its effects on recruited and infiltrating peripheral myeloid and lymphoid cells. For example, in $CD8^+$ T cells, AR signaling directly hampers IFN-γ/TNF secretion and cytotoxicity induced by T cell receptor engagement *in vitro* [59]. *In vivo,* experiments in sympathectomized mice revealed that β_2-AR signaling tempered systemic anti-viral $CD8^+$ T cell responses to influenza, most likely by hindering antigen-presenting cells, although this remains to be demonstrated

[60]. During infection with cytomegalovirus (CMV), NE synthesis by human neuronal cells was not affected [6] whereas β_2-AR activation down-regulated degranulation and IFN-γ production by Natural Killer (NK) cells, thus contributing to decreased murine resistance to CMV infection [19]. The involvement of adrenergic receptors in *T. gondii* infection has not yet been investigated.

Hence, beyond its key function in neurotransmission, the noradrenergic system can have a major effect on multiple immune cell types, both CNS-resident and peripheral. Activation of the *locus coeruleus* results in NE release that dampens neuroinflammation.

Can the enhanced neuroinflammation due to noradrenergic signaling inhibition explain the behavioral changes induced by *T. gondii* CNS infection?

Here, we propose that NE might act as a link between neurophysiological changes, neuroinflammatory processes, and the behavioral responses induced by *T. gondii* infection. Specifically, we hypothesize that the severe reduction in NE observed in the brains of *T. gondii*-infected rodents [6, 13], as opposed to the normal release of NE from the *locus coeruleus* in response to infection (**Figure 3A**), contributes to exacerbated neuroinflammatory responses in microglia, astrocytes, and possibly, recruited peripheral immune cells. In this model, elevated inflammatory cytokines such as IFN-γ, TNF-α and IL-6 (at least some of them) might induce the behavioral modifications associated with chronic *T. gondii* infection (**Figure 3, Key Figure**) [9]. The inflammation would be observed for the duration of the chronic infection. Of note, During chronic *T. gondii* infection in rats, there is 30-fold downregulation in dopamine-ß-hydroxylase (DBH) expression ; this limits NE release in response to infection [6]. We suggest that due to the deficient NE signaling, the normal inflammatory response to infection is amplified and these high concentrations of inflammatory cytokines might modulate the behavioral changes (**Figure 3B**). It has yet to be demonstrated that the elevated cytokine concentrations cause the behavior changes observed with *T. gondii* infection.

Several pieces of data lend support to our model. First, numerous reports find elevated inflammatory cytokines in the CNS during chronic *T. gondii* infection in rodents whereas NE concentrations are reduced [6, 22]. This is strengthened by an apparent inverse correlation between NE synthesis (based on *DBH* gene expression) and cytokine concentrations in *T. gondii*-infected rodents. That is, infection burden negatively correlates with NE production [6] whilst positively correlating with IFN-γ, TNF, and IL-12 concentrations [9] (**Table 1**). Shown are the Pearson's correlation coefficients for the correlation between brain cyst load and inflammatory cytokine or *DBH* mRNA. The inverse relationship supports their connection, although the link is indirect and both CNS NE expression and cerebral cytokine concentrations need to be tested in the same infected animals [9]. Second, systemic (intraperitoneal) administration of the AR agonist guanabenz, was reported to reverse *T. gondii*-induced hyperlocomotion in chronically-infected mice, independently from its parasiticidal effect [61]. The authors found that guanabenz restored normal locomotor activity in both encephalitis-

resistant (BALB/c) and encephalitis-susceptible (C57BL/6) mice, while guanabenz treatment only reduced brain parasite burden in BALB/c mice, implying an alternative mode of action on hyperlocomotion than directly reducing parasite counts [61]. Guanabenz, similar to other adrenergic agonists, binds to and activates α_2-Ars, hence simulating the action of NE on cells containing this receptor [62]. Indeed, guanabenz has a greater affinity for α_2-ARs than NE (ref again). Inflammatory cytokine production by microglia, astrocytes and invading immune cells is suppressed by α_2-AR agonists (**Figure 3C)** [63]. Furthermore, as well as modulating neurotransmission, guanabenz's immunomodulatory effects are highlighted by its systemic action on **TLR signaling** in mouse dendritic cells [64] and down-regulation of GM-CSF, IL-6, TNF, and IL-1β production in mouse macrophages [65]. Thirdly, this hypothesis is supported by providing a plausible mechanism for how the host behavior manipulation evolved. In this case, *T. gondii* promoting an immune response to suppress cyst reactivation and maintain a long-lasting infection (with little detriment to the host) could be a selective advantage for the parasite, contributing to parasite persistence. As down-regulation of NE permits elevated inflammatory responses, this in turn might aid in the suppression of parasite reactivation. We propose that the ensuing neuroinflammation might secondarily lead to altered host behavior.

Concluding remarks

T. gondii brain infection elicits global changes in neuronal function and consistent behavioral alterations, despite a seemingly stochastic infection of a limited number of neurons. We propose that (at least some of) the behavioral changes might be caused by excessive neuroinflammation; these might be unregulated due to the suppression of noradrenergic signaling that would normally limit inflammation during infection. Future and robust investigations are needed to clarify the mechanism(s) responsible for DBH down-regulation that reduce NE concentrations in the CNS following *T. gondii* infection. An essential question is whether the proposed model might also be applicable to other chronic CNS infections. Theoretically, this might be possible because other CNS pathogens such as Zika and West Nile viruses, also elicit neuroinflammation and cognitive alterations, although careful investigations are evidently warranted [52]. Although, *in vitro* experiments did not find DBH down-regulation with human CMV infection [6] other means of regulating noradrenergic signaling might be affected by other pathogens.

As future prospects, a systematic assessment of the effects of adrenergic modulators on cognitive alterations and inflammatory parameters might help classify any behavioral anomalies with *T. gondii* infection as dependent or independent from neuroinflammation (see **Outstanding Questions**). Further deciphering the inflammatory mediators and cellular players involved in the noradrenergic-neuroinflammation cross-talk might also help in the design of more targeted pharmacological approaches; ideally to alleviate behavioral aberrations caused by *T. gondii*, and potentially other CNS pathogens.

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Table 1. Both inflammation and decreased CNS NE synthesis correlate with *T. gondii* cyst load in the brain

Parameter measured	Correlation coefficient (r)	p value	Correlation curve equation	Reference
Dbh mRNA	-0.97	0.000017	y = -0.0358x + 0.3281	[6]
IFN-γ protein	0.77	0.012	y=0.365x+2.33	[9]
TNF protein	0.81	0.004	y=0.371x+2.34	[9]
IL-12 protein	0.6	0.021	y=0.311x+2.34	[9]

BOXES

Box 1. *Toxoplasma gondii*: a widespread and persistent brain parasite

Toxoplasma gondii (*T. gondii*) is a food-borne, zoonotic, protozoan parasite able to infect all warm-blooded animals. Upon infection of intermediate hosts (including rodents and humans), with environmental cysts or infected tissue, *T. gondii* convert to rapidly-dividing tachyzoite stages that infect and proliferate in intracellular parasitophorous vacuoles. During the acute phase, tachyzoites disseminate to tissues throughout the body, triggering strong **type 1 immune responses** that typically involve the IL-12/IFN-γ/TNF axis [34, 66]. These cytokines induce cell-autonomous defense pathways in phagocytes and promote the generation of cytotoxic $CD8^+$ T cells that directly target parasite-infected cells. These processes are essential for controlling acute infection but usually are insufficient to clear the parasite. The infection is maintained due to various effector proteins that modulate host cell responses [67]. Infection can persist for the host's entire lifetime, in the retina, muscles, and CNS. In these tissues, *T. gondii* forms cysts that contain the slower-growing bradyzoite stages [68] (**Figure 1**). The entire life cycle is maintained by passage from warm-blooded animals to felines, the only host capable of reproducing the sexual stages, by predation.

In the CNS, *T. gondii* can interact with various cell types, but cysts develop only in neurons [39]. The pathology varies in mice based on parasite strain and mouse species whereas rats are robust to infection with minor pathology. In humans, depending on the individual's ability to produce type 1 immune cytokines and robust T cell responses, the outcome of toxoplasmosis varies from an asymptomatic/mildly symptomatic latent infection to encephalitis with seizures to a life-threatening pathology. In the case of immune suppression (e.g. HIV/AIDS), parasite reactivation in the CNS can lead to a deadly neuroinflammatory disease called encephalitis [34, 66]. Primary exposure to *T. gondii* during pregnancy is a risk factor for fetal abnormalities or premature death.

Infection of humans with *T. gondii* is quite common, with a seroprevalence ranging from 10% to more than 50% depending on the geographic area [69]. The Centers for Disease Control (CDC) estimates that over 2 billion individuals worldwide carry the parasite, including more than 40 million people in the USA; the CDC classify this infection as a Neglected Parasitic Disease, given its high prevalence, potential severity, and the lack of curative therapeutics [70].

T. gondii infection has been associated with several behavioral aberrations such as altered locomotion and loss of predator fear and anxiety in rodents. In humans, *T. gondii* seropositivity correlates with schizophrenia [16] as well as with other neuropsychological disorders [15].

Box 2. Noradrenergic signaling and the CNS immune response

Norepinephrine (NE) is a well known neurotransmitter and hormone that in the mammalian brain acts on mood, concentration, learning, movement, sociality, alertness, and systemically modulates metabolic processes. NE contributes to fear memory in the cortex and amygdala, and NE blockers impair innate and contextual fear learning to cat odor in rodents. Aversive stimuli induce NE release that, in turn, enhances fear memory consolidation [6, 13]. To exert its effects, NE binds to G-protein coupled adrenergic receptors (ARs) receptors found on many cell types.

ARs are classified into two types, α and β, with two α sub-types and three β subtypes. α_1 receptors have a higher affinity for NE than adrenaline and are best known for stimulating muscle contraction and vasoconstriction. α_2 receptors are involved in feedback regulation of NE release by neurons and suppress intracellular cAMP [6, 13]. Major depression and schizophrenia are associated with α_2 adrenergic receptors and serve as a target for therapeutics [71]. Activated β-adrenoreceptors cause vasodilation in the circulatory system and increase intracellular cAMP activity. In the CNS, NE acts both as one of the primary neurotransmitters with roles in stress responses, anxiety, learning, memory, and movement, and as a regulator of neuroinflammation [6, 13]. In the brain, released NE may transduce signals in astrocytes, oligodendrocyte precursors, microglia, and neurons. β-ARs, mainly of the β_1 sub-type, are abundant on astrocytes [6, 13]. The β_1 receptor is also expressed at low concentrations in microglia and neurons. β_2 ARs are found in astrocytes, microglia, adult neurons, and oligodendrocytes [6, 13].

ARs display differences in their affinity for NE as well as for α and β-specific agonists and antagonists that are used to selectively manipulate noradrenergic signaling. “Beta blockers” are well known β-AR antagonists used systemically to reduce blood pressure that bind ARs in the heart and vasculature [72]. Agonists of the α_2-AR, including guanabenz, exhibit a higher affinity for these receptors than NE and act to mimic the action of NE [62, 63].

Glossary

Toxoplasma gondii:
Zoonotic intracellular parasite that infects warm-blooded animals including ~30% of the human population, classified as a neglected parasitic infection.

Tachyzoite:
The crescent-shaped rapidly replicating *T. gondii* stage that invades nucleated cells in the warm-blooded host, proliferates in intracellular vacuoles and disseminates during acute toxoplasmosis. These stages promote a strong immune response.

Bradyzoite:
The slowly growing and metabolizing chronic life cycle stage of *T. gondii* found in muscle and the central nervous system, within neurons, that forms a tissue cyst inside its host cell with hundreds to thousands of bradyzoites within a cyst that can persist for the host's lifetime (reviewed in [68]). A continuous immune response is required to prevent these from converting back to tachyzoites.

Type I, II, and III *Toxoplasma gondii*:
Type I, II and III strains represent the 3 main clonal lineages of *T. gondii*. Strain-specific polymorphisms in virulence factors affect the nature of the host immune responses. In laboratory mice, type I parasites are the most virulent while type II and III are less virulent, produce cysts and lead to chronic infection.

Neophobia:
The fear of anything new or novel. With rodents, the fear of new or novel situations or objects and a common behavioral test.

Host manipulation:
The hypothesis that a parasite or infecting organism alters its host animal specifically to the advantage of the parasite, such as increasing its transmission efficiency, by modifying behavior and/or host physiology.

Risk-aversive behavior:
To behave in a manner that avoids dangerous or risky situations such as avoiding predators.

Neuroinflammation:
Activation of immune responses in the brain and spinal cord, initiated in response to infection, injury, toxic metabolites, or autoimmunity.

Dendritic arbor:
The tree-like network of neuron extensions with a branching structure that send and receive signals.

Glia / glial cells:
Consisting of microglia, astrocytes, and oligodendrocytes, these are the non-electrical cells of the nervous system, which maintain homeostasis and protection of the neurons, and ensure potent innate immune responses in the brain.

Norepinephrine (NE):
Also known as noradrenaline, is one of the primary neurotransmitters. NE in the CNS is associated with stress, regulating anxiety, learning, memory, and movement.

Locus coeruleus (LC):
Brain region comprising the main cluster of NE-producing neurons in the CNS, with efferents extending throughout the brain and spine.

Dopamine ß-hydroxylase (DBH):
Enzyme responsible for NE synthesis by metabolism of dopamine within synaptic vesicles.

Adrenergic receptors (α/β):
G protein-coupled receptors that bind norepinephrine, stimulating the sympathetic nervous system; targeted by α_2 (e.g. Guanabenz) and β_2 agonist ligands.

Guanabenz:
Selective α2 adrenergic agonist with anti-inflammatory properties, used in the clinic against hypertension

Type 1 immune response:
These immune reactions are typically mounted in response to intracellular microbes. They comprise T-bet$^+$ IFN-γ–producing innate (e.g. innate lymphoid cells, ILC1) and adaptive cells (e.g. CD4$^+$ Th1 and CD8$^+$ Tc1) that protect the host through direct cytotoxicity and/or activation of mononuclear phagocytes.

Dendritic cells (DC):
Cells efficient at priming T cell responses through antigen presentation, co-stimulation and delivery of T cell-polarizing cytokines.

CD8$^+$ T cells:
Also known as cytotoxic T lymphocytes, MHC class I-restricted T cells that, upon activation, become equipped to produce IFN-γ, TNF, and cytotoxic mediators.

CD4$^+$ T cells:
Also known as helper T cells, MHC class II-restricted T cells that, upon activation, display regulatory (Tregs) or effector functions, sustaining cytotoxic CD8$^+$ T cell activity and memory formation, as well as humoral responses.

Interferon gamma (IFN-γ):
Inflammatory cytokine with a key protective function during *T. gondii* infection, produced by NK and T lymphocytes in response to IL-12, activates anti-microbial mechanisms in DC/macrophages, and boosts MHC I and II expression.

Granuloma:
Focal aggregate of immune cells that forms in response to a persistent inflammatory stimulus. Such structures have been described around tachyzoites in the brains of encephalitis-susceptible mice.

TLR signalling:
Toll-like receptors (TLR) are innate immune receptors that recognize different classes of pathogen-associated molecular patterns (nucleic acids, proteins, lipid moieties etc). TLR signaling plays significant roles in inflammation, immune cell regulation, survival, and proliferation.

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FIGURE LEGENDS

Figure 1. Schematic representation of the life cycle of *Toxoplasma gondii*. The feline host ingests the parasite when preying on a chronically-infected animal containing tissue cysts **(1**); sexual reproduction then begins within the feline gut endothelium **(2**). Oocysts are shed in the feces and persist in the environment (**3**) where they may be ingested by rodents and other warm-blooded animals (e.g. livestock). After ingestion, parasites differentiate into tachyzoites that proliferate in tissues (**4**). While the tachyzoites are cleared by the immune system, parasites invade muscle and brain and convert into slow-growing tissue cyst stages called bradyzoites. Humans can become infected by consuming undercooked meat (containing tissue cysts) or by ingesting oocyst-contaminated soil or food **(5**). Congenital transmission in humans and livestock can cause pregnancy complications **(6).**

Figure 2. Schematic overview of norepinephrine (NE) synthesis in a rodent brain.
NE is synthesized from dopamine that is 1) metabolized from tyrosine via L-DOPA, 2) imported into presynaptic vesicles by vesicular monoamine transporter (VMAT) and 3) converted into NE by dopamine-ß-hydroxylase (DBH). After release, NE binds α- and β-adrenergic receptors (ARs) and reuptake occurs via the norepinephrine transporter (NET). Noradrenergic neurons are principally located in the *locus coeruleus* (LC), with efferents projecting to most brain regions, including the hippocampus, medial prefrontal cortex (MPC) and amygdala.

KEY FIGURE

Figure 3. A model of noradrenergic regulation of the inflammatory response in the rodent brain during infection. (A) The normal functioning *locus coeruleus* releases norepinephrine (NE, triangles) that binds to adrenergic receptors on neurons, astrocytes, microglia, and immune cells. NE activates GABAergic neurons and microglia to repair neuronal dendrites and down-regulates pro-inflammatory cytokine release [56]. (B) During chronic *T. gondii* infection in rodents, neurons have been observed to exhibit dendritic spine loss [32], redistribution of GABA synthetic enzyme GAD67 [27], and severely decreased dopamine-ß-hydroxylase (DBH) and its product NE [6], whilst astrocytes exhibited decreased glutamate transporter Glt-1 [26]. Inflammatory cytokines TNF, IFN-γ, and IL1β are found to be elevated [9]. In our model, increase of cytokine concentrations is unrestricted by noradrenergic signaling during infection, that contribute to behavior changes observed in infected rodents. (C) We propose that the treatment of infected animals with the noradrenergic agonist guanabenz, that was found to reverse the hyperlocomotion induced in *T. gondii*-infected mice [61], (at least partially) compensates for the lack of NE and suppresses the inflammatory cytokines.