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# Neuroimmunology of rabies: New insights into an ancient disease

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## Abstract

Rabies is an ancient neuroinvasive viral (genus *Lyssavirus*, family *Rhabdoviridae*) disease affecting approximately 59,000 people worldwide. The central nervous system (CNS) is targeted, and rabies has a case fatality rate of almost 100% in humans and animals. Rabies is entirely preventable through proper vaccination, and thus, the highest incidence is typically observed in developing countries, mainly in Africa and Asia. However, there are still cases in European countries and the United States. Recently, demographic, increasing income levels, and the coronavirus disease 2019 (COVID-19) pandemic have caused a massive raising in the animal population, enhancing the need for preventive measures (e.g., vaccination, surveillance, and animal control programs), postexposure prophylaxis, and a better understanding of rabies pathophysiology to identify therapeutic targets, since there is no effective

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treatment after the onset of clinical manifestations. Here, we review the neuroimmune biology and mechanisms of rabies. Its pathogenesis involves a complex and poorly understood modulation of immune and brain functions associated with metabolic, synaptic, and neuronal impairments, resulting in fatal outcomes without significant histopathological lesions in the CNS. In this context, the neuroimmunological and neurochemical aspects of excitatory/inhibitory signaling (e.g., GABA/glutamate crosstalk) are likely related to the clinical manifestations of rabies infection. Uncovering new links between immunopathological mechanisms and neurochemical imbalance will be essential to identify novel potential therapeutic targets to reduce rabies morbidity and mortality.

#### KEYWORDS

neglected diseases, neuroimmunology, rabies

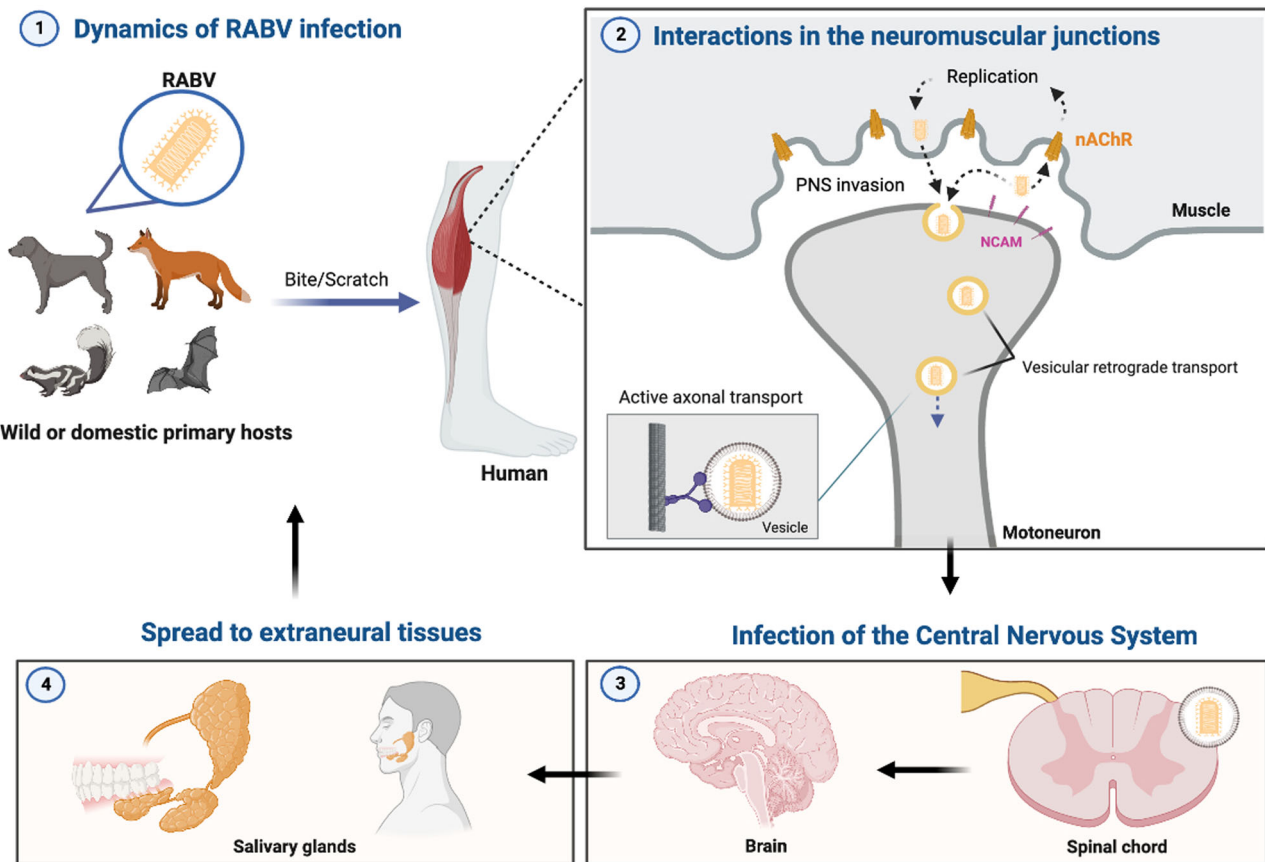
## 1 | INTRODUCTION

*Rabies lyssavirus* (RABV—Order *Mononegavirales*, Family *Rhabdoviridae*, genus *Lyssavirus*, Species *Rabies virus*) is a neurotropic single-stranded, negative-sense RNA virus (ssRNA-) that is the causative agent of rabies, an acute and highly lethal form of encephalomyelitis that affects humans and other mammals.<sup>1</sup> Rabies is a neglected disease, mainly affecting people living in communities of Africa and Asia, and to a lesser extent in the Americas.<sup>2–4</sup> However, the virus is also responsible for reports of human and animal infections across Europe and North America.<sup>5</sup> The circulation of RABV in wildlife makes eradication and surveillance challenging in high- and low-income countries.<sup>3</sup> Remarkably, the expansive growth of animal populations worldwide during the COVID-19 pandemic highlights the need to promote strategies supporting the prevention and control of rabies.<sup>6–8</sup> Furthermore, the increased risk of transmission to humans highlights the crucial need to better understand the mechanisms of RABV pathophysiology, as there is still a lack of effective treatment beyond prevention and postexposure prophylaxis. The disease's kinetics involves a complex disturbance in the neurotransmission signaling by disrupting gamma-aminobutyric acid (GABA) levels in the brain,<sup>9–11</sup> an essential neurotransmitter also involved in brain metabolism and the neuroimmune response,<sup>12</sup> which has been reviewed elsewhere.<sup>13</sup> GABA is a central neurotransmitter with neuroimmunological inhibitory properties and is also produced by immune cells, which in turn express GABA receptors (GABAR).

Notably, the interference with GABA-GABAR interaction is not the only neuroimmunological aspect of RABV infection. RABV also interacts with some classes of neurotransmitter receptors expressed in the neural cells of the peripheral nervous system (PNS), which is mediated by RABV glycoprotein (G-protein) (Supplementary Information I), and subverts the infiltrative and resident immune defenses to preserve its main cellular reservoir: the neurons.<sup>14–16</sup> The main targets are ionotropic acetylcholine (ACh) receptors, such as nicotinic receptors (nAChR) in the neuromuscular junction (Figure 1), neurons,

and immune cells. The nAChR are ionotropic receptors (ligand-gated ion-channel) that mediate the neural signaling at the muscle in response to ACh, mainly released by the autonomic nervous system.<sup>17</sup> Interactions with the viral G protein and ACh nicotinic receptor nAChR- $\alpha 4\beta 2$  distributed in some specific brain areas may be related to rabies-induced behavioral disorders in the host. These interactions may cause serotonergic-related motor alterations evoked by the disruption of cholinergic signaling.<sup>18</sup> Although RABV affects multiple neurotransmitter systems, the exact mechanisms and the sequence of events leading up to the severe clinical manifestations are poorly understood.

Another neuroimmunological aspect of the RABV infection could potentially connect the molecular mechanisms of RABV and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These two viruses use metabotropic glutamate receptors (mGLURs) as internalization factors to infect host cells,<sup>19,20</sup> reducing the protein expression in the cell membrane. mGLURs belong to the G-protein coupled receptors (GPCR) family and regulate various neural functions, including the control of presynaptic neurotransmitter release.<sup>21</sup> These receptors are divided into three classes, including group I (mGLUR1 and mGLUR5), II (mGLUR2 and mGLUR3), and III (mGLUR4, mGLUR6–8). Interestingly, previous studies have described the expression of classes I and II mGLURs in immune cells and their role in immune functions.<sup>22,23</sup> For instance, the activation of class I mGLUR induces neutrophil migration toward the injury/infection site by stimulating intracellular pathways related to  $\beta 2$  integrin transduction.<sup>24</sup> This finding indicates the involvement of glutamate as a positive regulator of chemotaxis. In turn, Pacheco et al.<sup>25</sup> found that mGLUR5 is constitutively expressed by CD4<sup>+</sup> and CD8<sup>+</sup>-T cells, whereas mGLUR1 is only expressed by activated CD4<sup>+</sup> and CD8<sup>+</sup>-T cells. The authors indicate a dual role of glutamate in the T cell response since mGLUR5 downstream inhibits the proliferation in a G-stimulatory protein-dependent manner (Gs). However, this event is abolished when mGLUR1 is functionally expressed, suggesting the role of the latter receptor in activating T cells entering the nervous system.



**FIGURE 1** *Rabies lyssavirus* (RABV) dynamics: from the initial infection to neuroinvasion in the host organism. From 1 to 4, it shows that once inoculated, RABV replicates in the muscles after the interaction with nicotinic receptors (nACh) and invades the peripheral nerves that transport the virus toward the central nervous system (CNS). RABV also infects extraneural tissues, such as the salivary glands, facilitating its spread to other naive hosts. nAChR, nicotinic receptors; NCAM, neural cell adhesion molecule. Created with [biorender.com](https://www.biorender.com).

A solid neuroimmune intersection exists in rabies, and we hypothesize that several other neuroimmune biological aspects of this viral infection remain to be investigated. Future works deepening our understanding in this regard might reveal several new interactions not only during rabies physiopathology but also for other illnesses and possibly autoimmune diseases targeting the nervous tissue. Here, we aim to review and summarize the current knowledge of neuroimmunological aspects of rabies, demonstrating how immune and glial cells respond to neuroinvasion. Further, we aim to discuss the mechanisms by which RABV subverts the immune machinery and disturbs neuronal physiology while systematically affecting the immune response.

## 2 | GENERAL ASPECTS OF RABIES

### 2.1 | RABV dynamics in humans and nonhuman hosts

The RABV has evolved molecular mechanisms that confer structural properties for the virion and are fundamental for replication and cellular invasion within the central nervous system (CNS). RABV

encodes five proteins (N, L, P, M, and G) in its genome<sup>26</sup> (Supplementary Figure). The nucleoprotein (N) encapsulates the viral genome, forming the ribonucleoprotein (RNP) complex; the viral transcription and replication are managed by the polymerase (L) and its cofactor, the phosphoprotein (P); the matrix protein (M) connects the nucleocapsid with the envelope.<sup>27</sup> Finally, the glycoprotein (G), a surface protein, plays a crucial role in cell invasion, as it interacts with cellular receptors to mediate viral entry into the cell.<sup>28</sup>

Rabies transmission usually occurs upon contact with infected saliva from bites or scratches of infected animals, such as bats, dogs, skunks, and foxes (Figure 1).<sup>1</sup> Although extremely rare, viral transmission may occur following organ donation<sup>29</sup> or inhalation of aerosol.<sup>30,31</sup> After invading the host, the RABV can undergo a short period of replication in the muscle tissue before migrating to the CNS. However, this replication period can be brief, depending on the viral titer in the inoculum,<sup>32</sup> the local of exposure, or a direct neuronal infection may occur without prior replication in the muscle.<sup>1,33</sup> After replicating in muscle, the rabies virus crosses the neuromuscular junction and binds to various cellular receptors, going from the peripheral nervous system (PNS) to the CNS.<sup>1,32</sup> The most well-characterized receptor in rabies neuronal invasion is the nAChR.<sup>18,28,34</sup> However, other host receptors or attachment factors

are possibly involved in RABV invasion, such as the mGLUT2R, the low-affinity nerve growth factor receptor (p75NTR), the neural cell adhesion molecule (NCAM), integrin  $\beta 1$ ,<sup>35</sup> and heparan sulfate.<sup>36</sup>

Currently, the most effective way of preventing rabies is the administration of pre- or postexposure human vaccination and monitoring infected domestic animals.<sup>37</sup> However, once the virus reaches the CNS, it causes a lethal disease with progressively severe neurological symptoms. The incubation period is highly variable, ranging from weeks to years,<sup>38</sup> although the signs usually manifest between 15 and 30 days postinfection in humans. Initially, nonspecific symptoms such as headache and fever are observed. Classic manifestations, such as hydrophobia, hypersalivation, paresthesia (numbness or tingling sensations), and agitation, can occur<sup>39</sup> as the disease progresses.

Despite the CNS being the origin of clinical symptoms, RABV physiopathology is not limited to infection of the CNS. RABV spreads centrifugally to extraneural tissues and can reach the salivary glands of humans and animals.<sup>1,27</sup> Due to these characteristics, the RABV has assured its permanence in nature for roughly four millennia.<sup>40</sup>

Currently rabies is entirely preventable with proper postexposure prophylaxis (PEP), which is given to 15–29 million patients exposed to rabies each year.<sup>41</sup> However, rabies is almost 100% fatal after the onset of clinical signs. Some reports of patients that survived after clinical disease,<sup>42,43</sup> and preclinical studies recently described a potential therapeutic approach using monoclonal antibodies. However, despite being known for a long time, an efficient treatment against symptomatic rabies is not yet available.<sup>44</sup> The high mortality rate of rabies results from the lack of a well-characterized therapeutic protocol, which makes it challenging to eradicate the disease. In addition, many aspects of its physiopathology remain unclear.

## 2.2 | Rapid spreading

Rabies virus widely disseminates throughout the CNS long before patients or naturally infected dogs exhibit clinical symptoms. The time of viral propagation in the CNS is constant, but the period of time the virus stays at the inoculation site varies (depending on the state of the smoldering infection).<sup>45</sup> Local neuropathic pain at the inoculation site may occur, although the earliest pathogenic event generally considered is the temporal sequence of virus outflow to the sensory ganglion (SG) and local lymph node.<sup>46</sup> Activated T cells then attack SG resulting in inflammation and pain. Hence, interventions to block entry and dissemination must initiate before the virus gains access to the nerve ending, not after symptoms have appeared. The virus can spread over the central neural axis within a few days.<sup>47</sup> Viral propagation to the CNS occurs along the sensory nerve from the infection site resulting in viral dissemination throughout the CNS, followed by centrifugal spread to SG. From there, the RABV travels to lymph nodes at the corresponding region. In this context, antibody production and trafficking of activated T cells have been associated with dorsal root ganglionitis.<sup>48</sup> This may explain why antibodies,

specifically neutralizing antibodies, appear late, mainly in the systemic circulation, not in the CSF. Moreover, cellular immune reaction occurs only when the virus moves out from the CNS and can be immunologically recognized.<sup>49</sup>

## 2.3 | The immune biology of RABV infection

Triggering an early and fast immune response against RABV is essential for developing an efficient pathogen-specific immunity that prevents the virus from reaching the CNS. However, when RABV reaches the CNS, the virus exploits evasive strategies to escape the immune response in a cell-specific manner.<sup>50</sup> Infections that target the brain parenchyma are naturally favored by the immune-privileged aspect of the CNS because the immune system, due to its high adaptive and homeostatic capacity, approach the infection in a less invasive and aggressive way to attenuate the harmful effects of antiviral response. This process is essential for RABV to establish a productive infection in the brain. In this context, the more pathogenic the RABV strain is, the less it evokes an acute and robust immune response.<sup>51</sup>

The RABV particles are widely recognized in the periphery by pattern-recognition receptors (PRR) expressed by recruited phagocytes, which sense pathogen-associated molecular patterns (PAMPs) through toll-like receptors (TLR).<sup>52</sup> However, RABV suppresses locally the signaling elicited by immune cells by interfering with the type-I IFN- $\alpha$  and IFN- $\beta$  induction. The virus achieves this through its phosphoprotein (P), which inhibits the phosphorylation of the interferon regulatory factor 3 (IRF-3). This delays the antiviral and adaptive immune response triggered by many interferon-stimulated genes (ISGs).<sup>53</sup> This strategy is crucial for establishing CNS infection.<sup>54</sup> Moreover, different classes of PRRs may induce divergent responses. Chen et al.<sup>55</sup> suggested that TLR4, but not TLR2, is indispensable for humoral immunity induction after live-attenuated rabies vaccination. The activation of TLR4 elicits a type-2 conventional dendritic cell response (cDC2) in the spleen and other lymphoid organs, increasing the differentiation of T-helper follicular cells (Tfh) and the formation of germinal centers (GC), leading to antibody production by B-cells. Luo et al.,<sup>56</sup> highlighting the role of TLR7 in RABV-specific antibody production, demonstrated that TLR7 facilitates several immunological events such as GC formation, B-cells recruitment to GC, induction of Th1-related molecules (e.g., IFN- $\gamma$  and interleukin [IL]-6), and promotion of long-term immunity after RABV vaccination.

During natural infection, the host fails to generate early virus-neutralizing antibodies (VNA) and can thus not resist the fatal outcome.<sup>57,58</sup> Some hypotheses may explain this event. For instance, Yang et al.<sup>59</sup> suggested that the blockage of DC-mediated immunity could be a critical event. This group demonstrated that the wild-type (wt) strain failed to elicit DC activation due to the weak binding of RABV to DCs in a viral G-protein-dependent manner and limited synthesis of its RNA. Although it may partially explain the delaying humoral response during RABV infection, evaluating and comparing



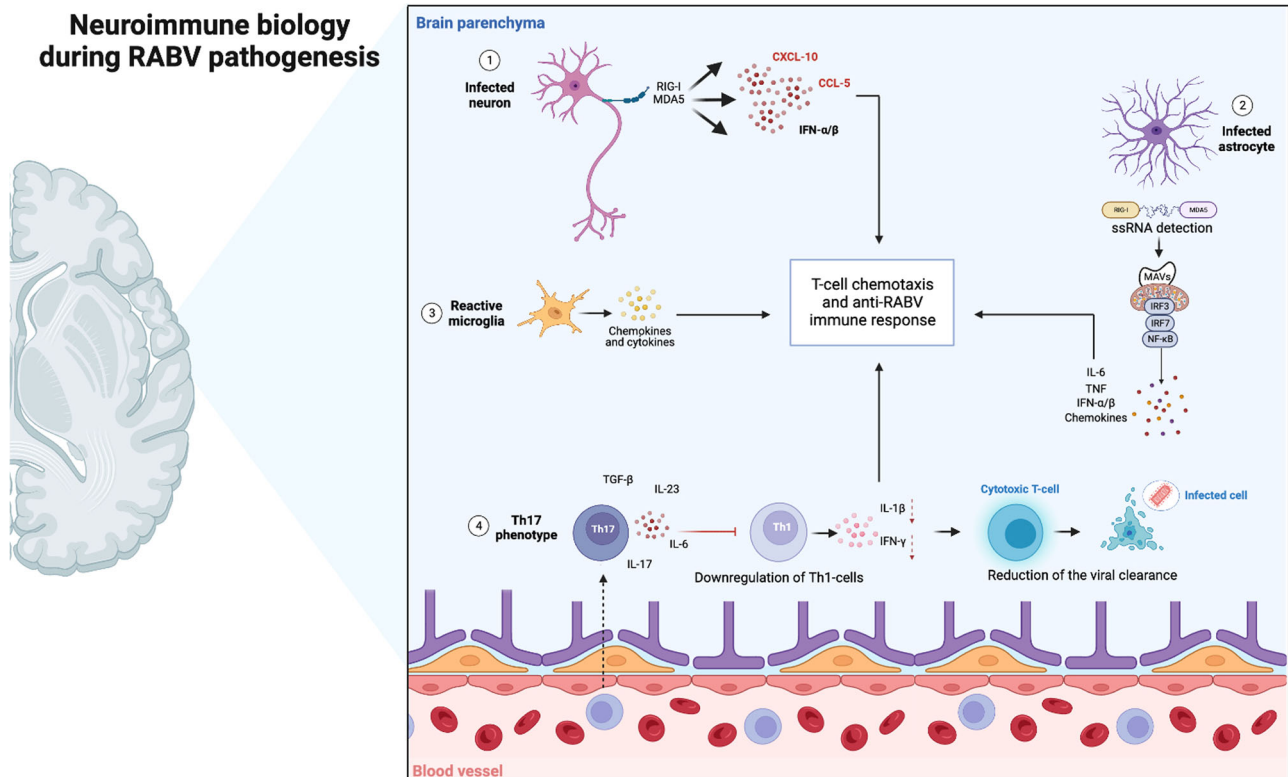
how different RABV strains (e.g., bat-isolated strains) cause variable disease phenotypes would be interesting.

Similarly to DCs, macrophages are other early antigen-presenting cells (APC) that interact with RABV locally, as shown by Embregts et al.<sup>60</sup> The authors demonstrated that the wildtype, street RABV, promotes a nicotine-like effect by activating the cholinergic anti-inflammatory pathway (CAP) in macrophages. It is initiated by the interaction of the viral glycoprotein with the nACh- $\alpha 7$  receptors expressed in these cells. The CAP activation causes NF- $\kappa$ B retention in the cytoplasm and reduces the release of pro-inflammatory cytokines by evoking IL-10 production and T-cell suppression *in vitro*. The authors proposed that suppressing the local immune response by polarizing the innate cells to an anti-inflammatory phenotype could be part of the evasive strategy of RABV to subvert and delay a systemic immune response.

Upon neuronal infection, PRRs expressed in neurons can sense viral products (e.g., viral proteins and ssRNA) following the trans-synaptically RABV spread within the parenchyma. In this local, the antiviral response is characterized by the production of type I IFNs and other cytokines (e.g., Th17 and Th1),<sup>61</sup> as well as chemokines that attract leukocytes from the periphery (Figure 2).<sup>62</sup> Chai et al.<sup>14</sup> highlighted that the neuron release of the chemokine CXCL10 is a

crucial factor during RABV neuroinvasion because it acts in the chemoattraction of CXCR3<sup>+</sup> CD4<sup>+</sup>-T cell, modulation of the blood–brain barrier (BBB) permeability, and paracrine signaling to resident cells. This is of interest since Choppy et al.<sup>15</sup> suggested the controversial role of innate immunity during the pathogenesis of rabies. Using a transgenic model of mice over-expressing the LGP2 protein, a negative regulator of the RIG-I-mediated innate immune response, they demonstrated a reduction in morbidity in mice expressing LGP2. The reduced expression of immune molecules, such as IFN- $\beta$ , favors T-cell infiltration in the brain parenchyma because the latter increases the expression of the regulatory B7-H1 molecule, which promotes CD8<sup>+</sup>-cell apoptosis, thus contributing to RABV neuroinvasiveness.<sup>63</sup> Those studies highlight the complexity of the immune signaling, crosstalk (Table 1, Supporting Information), and the mechanisms RABV exploits to interact with immune surveillance. Therefore, even though the glial and peripheral immune cells respond to the infection in an attenuated manner, the innate and adaptive antiviral mechanisms are insufficient to prevent the death of the host.<sup>66</sup>

Notably, postmortem studies of naturally infected dogs at an early stage of infection revealed insufficient inflammatory responses as determined by measurement of cyto-chemokine, transcripts.<sup>67</sup> The



**FIGURE 2** Neuroimmunological events of rabies. Several immunological phenomena occur in the central nervous system (CNS) during the antirabies immune response, including the production of cytokines and chemokines by neurons, astrocytes, microglia, and local immune cells (steps 1–3). The immune mediators produced locally modulate blood–brain barrier (BBB) permeability and chemotaxis, essential to mounting an antirabies immune response, such as activating adaptive immune (e.g., cytotoxic T cells). CCL, CC chemokine ligand; CXCL, chemokine (C-X-C motif) ligand; IL, interleukins; IN, interferons; IRFs, interferon regulatory factors; MDA-5, melanoma differentiation-associated protein 5; RIG-I, retinoic acid-inducible gene I; ssRNA, single-stranded RNA; TF, tumor necrosis factor; TGF-3, transforming growth factor beta. Created with [biorender.com](https://biorender.com).

**TABLE 1** Immunological profiling and in situ immune interactions during *Rabies lyssavirus* (RABV) neuroinvasiveness.

Authors	Main findings	Study type
Feige et al. <sup>64</sup>	wt RABV changes the basal expression of <i>Ikkb</i> , <i>lfnar1</i> , <i>lfngr2</i> , and <i>CX3CR1</i> genes, which are modulated by CCR5, CCR3, interleukin (IL)-10 and interferons. It triggered the expression of the CXCL-16 chemokine, changing the dynamics of immune mediators and neurotransmitter release.	Postmortem, in vivo (BALB/c mice) and in vitro (Murine cortical astrocytes)
Santos et al. <sup>61</sup>	Th17 immune profile—characterized by the expression of the cytokines TGF- $\beta$ , IL-6, IL-23, and IL-17—is highly expressed in fatal cases of human rabies transmitted by dogs. The authors indicate it favors the virus to evade viral clearance since high levels of IL-23 downregulate the expression of the Th1-related cytokines such as IL-1 $\beta$ e IFN- $\gamma$ .	Postmortem whole brain analysis
Chai et al. <sup>14</sup>	Anti-CXCL10 antibodies reduced IFN- $\gamma$ production, Th17 profile cell infiltration, TJ protein expression, and decreased BBB permeability. High expression of CXCL10 was initially identified in neurons three days postinfection (dpi) in RABV-infected mice. After six dpi was detected in microglia and nine dpi in astrocytes. Therefore, CXCL10 initiates an immune cascade that activates microglia/astrocytes, inflammatory infiltrates, and cytokine and chemokine expression in the CNS.	In vivo (mice infected)
Tian et al. <sup>65</sup>	The astrocytes play a crucial role in regulating the BBB, the primary source of cytokines during viral neuroinvasion. Cytokine production depends on the MAVS signaling pathway during RABV infection in the CNS. The expression of RIG-I, p-IRF7, STAT1, and IFIT1 in astrocytes infected with the laboratory attenuated strain CVS-B2c (B2c) were higher than with RABV DRV-AH08 (DRV) wild strain. B2c strain positively regulates inflammatory cytokines in astrocytes, enhancing BBB permeability. The B2v strain activates the MAVS signaling pathway by viral RNA, causing the production of IFN and ISGs in astrocytes. It also triggers inflammatory cytokines production in astrocytes (TNF- $\alpha$ , IL-6, IL-1 $\beta$ , IFN- $\gamma$ , IL-17 e VEGF) which may cause BBB disruption.	In vivo (C57BL/6 and MAV-/- mice) and in vitro (primary mouse neurons and astrocytes)

Abbreviations: BBB, blood-brain barrier; CCR, chemokine receptor; IFN, interferon; IL, interleukin; MAVS, mitochondrial antiviral-signaling proteins; TJ, tight junction proteins.

degree of response, nevertheless, is more pronounced, although insufficient, in paralytic than furious rabies.<sup>46,67</sup> Cellular infiltration is seen only at the brainstem in paralytic rabies at an early stage.<sup>68</sup> This differs from the mice model and experiments using the fixed strain challenge virus standard (CVC-31).<sup>69</sup> However, positron emission tomography (PET) neuroimaging using translocator protein (TSPO) binding radioligand may be needed to obtain a broader view of the inflammatory process involved in rabies. TSPO at the outer mitochondrial membrane subserves functions of membrane synthesis and adjusts mitochondrial functions.<sup>70</sup> TSPO is highly expressed in activated microglia (a hallmark of neuro-inflammation),<sup>71</sup> and if detected by tracers would signify the activity of inflammation.

### 3 | ROLE OF NEUROGLIAL PATHOLOGY IN RABIES OUTCOME

#### 3.1 | Glial response to the infection

The brain's innate and adaptive immunity<sup>72</sup> is diverse, consisting of chemical and cellular components.<sup>73,74</sup> Thus, the brain is protected from pathogens primarily by resident glial cells (oligodendrocytes, astrocytes, and microglia) that outnumber the neurons. Under physiological conditions, the brain microenvironment generates signals that prevent astrocytic and microglial activation.<sup>75</sup> Furthermore, those cells interact with neurons, modulating their activity,

development, metabolism, and signaling.<sup>76</sup> These interactions between microglia, astrocytes, and neurons are essential for the function of innate immunity in the brain.<sup>77</sup> However, little is known about the role of glial cell communication during neuroinvasive infections like RABV<sup>78</sup> and the role glial cells play in rabies-induced neuropathogenesis.

Oligodendrocytes are myelin-producing cells of the CNS that facilitate the propagation of action potentials from axons. In the peripheral nervous system, they are called Schwann cells. In this context, the oligodendrocytes are critical for myelin regeneration following injury in the most demyelinating disease like multiple sclerosis.<sup>79</sup> Another function includes communication with microglia through exosome secretion that is internalized by microglia, avoiding the induction of inflammatory response by free exosomes in the turnover of the oligodendrocyte's membrane.<sup>80</sup>

Astrocytes represent the most abundant cell type in the CNS, playing an essential role in brain homeostasis.<sup>81</sup> In addition to monitoring brain development and function, astrocytes control potassium levels, remove toxic substances,<sup>82</sup> and modulate synaptic activity.<sup>83</sup> They also become reactive under diverse pathological conditions, acting at the beginning of the antiviral response through a type I IFN-dependent mechanism. Astrocytes also express class II major histocompatibility complexes (MHC-II) and costimulatory molecules.<sup>77</sup>

Unlike astrocytes, microglia represent a less abundant cell subpopulation, being cells derived from primitive myeloid progenitors

of the yolk sac in embryogenesis and an adult brain from macrophages, mainly residing in the gray matter.<sup>76</sup> These cells are designated as CNS resident macrophages.<sup>84</sup> Under normal conditions, microglia stay in a resting state (M0). However, microglia can acquire two central functional states in a neurological injury or infection: a pro-inflammatory (M1) and anti-inflammatory (M2) phenotype.<sup>85</sup>

Microglial cells with the M1 phenotype express mainly CD32, CD64, and CD86 receptors. These cells also produce IL-1 $\beta$ , IL-6, IL-12, and IL-23, besides tumor necrosis factor (TNF)- $\alpha$ , inducible nitric oxide synthase, and chemokine (C-C motif) ligand 5 (CCL5). In contrast, the M2-microglia phenotype is characterized by the upregulation of arginase (ARG)-1, mannose receptor (CD206), and insulin-like growth factor (IGF)-1, triggering receptor expressed on myeloid cells 2 (TREM2), chitinase 3-like 3 (Ym-1), and FIZZ1.<sup>86</sup>

Neuroinflammation relates to a pathological immune response from the brain, and ranges in severity, and can be acute or chronic.<sup>86</sup> In this context, most brain cells respond to inflammatory signals, including neurons, astrocytes, oligodendrocytes, microglia, and even the cells of blood vessels and the meninges.<sup>87</sup> Under neuroinflammatory or neurodegenerative situations, microglia are thought to contribute to pathogenicity. However, in the context of viral encephalitis remains unclear if it is beneficial or detrimental to disease outcomes.<sup>88</sup> In addition, other neurotropic viruses have also been described to induce microglia activation in CNS, including the flaviviruses Japanese encephalitis virus,<sup>89</sup> Dengue virus,<sup>90</sup> Zika virus,<sup>90</sup> and West Nile virus.<sup>88</sup> As the resident immune effector cells within the CNS, microglia cells are likely to encounter pathogens at various stages of infection. It is also reported that RABV infection may influence the microglial phenotype.<sup>50</sup>

Interestingly, as previously stated in the literature, there is an almost complete absence of inflammatory response in the CNS since preservation of the neuronal network is crucial for disease progression and viral transmission to other hosts.<sup>51</sup> Thus, RABV has a repertoire of evasion mechanisms to escape the host immune response by utilizing various strategies to evade detection by the host.<sup>91</sup> Indeed, models based on in vitro-infected astrocytes suggest that attenuated RABV triggers astrocyte inflammatory responses through viral recognition by retinoic acid-inducible gene I (RIG-I)/melanoma differentiation-associated protein 5. Meanwhile, wild-type RABV isolates can evade the host immune responses from astrocytes or other glial cells, allowing the virus to reach pathogenic levels.<sup>65,92</sup>

However, once in the CNS, the RABV triggers a RIG-I-mediated immune response in the neurons, astrocytes, and microglia. This results in a primary response mediated by type I-IFN that activates IRF3 and NF- $\kappa$ B. The type II-IFN response activates JAK-STAT signaling and IRF7 pathways, which leads to cytokine (IL-6, TNF- $\alpha$ , IL-12, and IL-5) release and antiviral state.<sup>32,93</sup> Following the infection, RABV is recognized by NLRP3 inflammasome and activates IL-1 $\beta$  release, possibly supporting the recruitment of inflammatory cells and control of viral dissemination since the absence of IL-1 $\beta$  receptors enhances viral pathogenicity.<sup>32</sup> Furthermore, the secretion of both cytokines and chemokines stimulates the expression of class I and II

MHC molecules, increases the expression of adhesion molecules, and promotes BBB opening.<sup>92</sup>

## 3.2 | Neuronal and cellular dysfunction

Despite the disease severity and lethality, the RABV infection of the parenchyma is not accompanied by significant macroscopic changes in the brain.<sup>94</sup> However, the disease's pathophysiology is characterized by in situ histological findings<sup>95,96</sup> that may differ in intensity depending on the virulence of the RABV variant<sup>97</sup> and the phase of infection in the brain and spinal cord.<sup>98</sup> Most brain metabolic and functional disorders occur in the late stage of the disease.<sup>99</sup> The histopathological changes in the brain are generally relatively mild, with inflammatory cell infiltration into the leptomeninges and mononuclear perivascular cuffing in the parenchyma. Remarkably, some morphologic changes in neuronal processes during RABV infection are similar to the neurodegenerative changes in diabetic sensory and autonomic neuropathy, such as the appearance of a "bifurcating" somata. This event indicates cytotoxicity and the presence of axonal swellings composed of accumulations of mitochondria<sup>100</sup> and cytoskeletal proteins.<sup>101,102</sup> Thus, these data based on in vitro and *postmortem* brain analysis suggest that the functional dysfunction under RABV infection plays a crucial role in the disease outcome rather than cell death.

A management protocol for the treatment of confirmed or suspected (human) rabies infections described selection criteria, paths to investigate, and combination use of several therapeutics, including broad-spectrum RNA-dependent RNA polymerase inhibitors.<sup>103</sup> A recent mini-review lists the use of cannabidiol in palliation with the potential to counter the damaging process.<sup>45</sup> Also, the use of Favipiravir<sup>104</sup> successfully rescued rabies-infected animals. The virus was conjugated by a tracer, demonstrating that animals could survive even when the virus had already reached the CNS.<sup>105</sup>

### 3.2.1 | Alterations in apoptosis

Fu and Jackson<sup>94</sup> concluded that despite the severe clinical signs of rabies and the severity of the disease, the fatal outcome in the late phase must be due to neuronal dysfunction, considering that electrophysiologic alterations are associated with disorders in ion-channel mediated signaling. This must play an essential role in cerebral failure as the disease progresses. For them, decreasing sodium and potassium channels could result in synaptic impairment since it might prevent neurons from generating action potentials and the subsequent release of neurotransmitters, leading to functional death. Interestingly, the virus exploits its ability to preserve neuronal machinery without causing apoptosis, cellular death, or other cytopathic effects. This is accomplished by promoting T-cell death to prevent viral clearance from the tissue and avoid leaving histopathological traces.<sup>106,107</sup> However, the precise role of cell death during the disease remains controversial.



Jackson et al.<sup>33</sup> argue that neuronal apoptosis is irrelevant during the natural (wildtype) RABV infection. In line with Jackson, Lafon,<sup>106</sup> and Fernandes<sup>108</sup> proposed that the virus uses anti-apoptotic strategies to preserve neurons and propagate the infection, enhancing proapoptotic pathways to subvert and attenuate the immune responses.<sup>107</sup> In contrast, Kip et al.<sup>109</sup> indicated the involvement of neuronal and macrophage apoptosis and pyroptosis in the onset of disease during ERA (lab-attenuated strain) or CVS-11 (high pathogenic lab-strain) infection in a caspase-3-deficient mouse (*caspase-3<sup>-/-</sup>*). Meanwhile, Peng et al.<sup>110</sup> indicated that besides apoptosis, M-protein-mediated autophagy also plays a crucial role during RABV-host interaction in the brain because this process may be protective under cellular infection-evoked stress. These differential effects during disease onset may differ according to the viral strain, experimental model, and RABV pathogenicity.

### 3.2.2 | Cytokine and chemokine

Furthermore, the increased production of cytokines and chemokines by neurons, glial cells, and peripheral leukocytes in response to neuroinvasion<sup>51</sup> or other neuroinflammatory conditions changes basal synaptic functions in different brain areas. For instance, the exposure of cortical neurons to IL-1 $\beta$  or TNF- $\alpha$  is associated with dendritic damage and loss, followed by increased production of both intracellular and extracellular glutamate and increased activity of glutaminases.<sup>111</sup> This event suggests the role of cytokine-triggered neurotoxicity in a model of HIV encephalitis.<sup>111</sup> In addition, Feige et al.<sup>64</sup> indicated that the chemokine CXC motif chemokine ligand 16 (CXCL-16) modulates both inhibitory pathways and excitatory signaling in hippocampal cells.<sup>112</sup> The authors suggested that CXCL-16, possibly released by microglial cells, increases the spontaneous GABA and glutamate release. They proposed that this results from glia-neuron crosstalk, involving the releasing of other neuromodulatory molecules (e.g., adenosine and CCL2). CXCL-10 is another important chemokine highly upregulated by neural cells in rabies.<sup>14</sup> Under neuroinflammatory conditions and upon chronic exposure, CXCL-10 increases protein expression of glutamate and GABA receptors by rat hippocampal neurons.<sup>113</sup> Most of these immunological mediators in rabies are produced by neural cells and leukocytes. Thus, there is probably a molecular neuroimmunological network that remains to be holistically explored, allowing us to further understand the etiopathology of rabies infection.

### 3.2.3 | Ionic dysregulation

Although not characterizing possible immunological implications, whole-brain proteomic analysis of mice infected with wt RABV results in differential modulation of host proteins engaged in neuronal homeostasis and synaptogenesis, such as proteins of the SNARE-complex (syntaxin-18,  $\alpha$ -SNAP, and TRIM-9) in different

stages of the disease.<sup>114</sup> This approach revealed upregulated levels of Na<sup>+</sup>/K<sup>+</sup>-ATPase and H<sup>+</sup>-ATPase, active pumps that maintain cell osmolarity, ion transport on the cell membrane, and neuron excitability (Figure 3).<sup>115</sup> Thus, increased expression of those transporters may be linked with neuronal dysfunction.<sup>116,117</sup> Furthermore, the whole-brain proteomic analysis<sup>114</sup> indicated a dysregulation of calcium signaling in RABV-infected cells, which was functionally confirmed by calcium-imaging tracing studied by Kim et al.<sup>10</sup> These researchers showed that RABV differentially downregulates a range of genes related to cellular and synaptic functions by activating Ras/Erk/MAPK signaling in an M-protein-dependent manner to mediate Ca<sup>2+</sup> downregulation. Reduction in the calcium concentrations negatively affects neurons' spontaneous activity, which was reported during the disease kinetics.<sup>10</sup> The authors have also pointed out the involvement of the GABAergic system (i.e., GABA<sub>A</sub>-receptor-activation), which displays a synergism in RABV-induced intracellular calcium decreases. Therefore, these data indicate the involvement of GABA and other GABA<sub>A</sub>-receptor agonists in the neuropathology of rabies. These functional changes in protein expression and ion homeostasis may affect the release of neurotransmitters, causing vesicular accumulation in the presynaptic cells<sup>114</sup> due to the low availability of Ca<sup>2+</sup> to promote the vesicular fusion through SNARE-complex (Figure 3)<sup>118,119</sup> neuron excitability.

### 3.2.4 | Preservation of neuronal and axonal function and integrity

In vivo studies of naturally infected dogs of both, furious and paralytic forms, during the early stage (still conscious) showed increased fractional anisotropy by diffusion tensor imaging, that is, preserved molecular transport or axon stills intact. Faster dissemination is evident in the case of furious compared to paralytic rabies in accord with *postmortem* findings, with a more increased intercellular spread in the former.<sup>67</sup>

The underlying mechanisms are autophagic flux in wild-type virus and neuronal cell reactivity, which is biased against apoptosis, thereby maintaining neuronal and axon functions and integrity.<sup>110</sup> Autophagy is incomplete (autophagosome not fusing with lysosome); thus, the process, instead of extruding the virus, then retaining the virus for further replicative cycles.<sup>120</sup> Mitochondrial activation is an upstream and also downstream event. Mitochondria senses danger signals via ER stress and begins the process of ubiquitination, unfolded protein response, and determination of autophagy bias as well as undergoing activities to maintain homeostasis of mitochondria dynamics.<sup>104</sup> A downstream event occurs when the capability to nurture the virus is no longer valid. Bioenergetic failure is the result, perhaps at this near- or terminal phase, when microcellular and microcellular damage manifest and axonal beading and signs of neurodegeneration take place. At this stage, symptoms start to appear. Treatment to block hazardous consequences and save mitochondria must be done during the early pathology phase.



In brief, RABV infection is followed by the downregulation and reduced protein levels of modulators of the synaptic, immune, and homeostatic functions. Besides, neuroinvasion and PAMP detection trigger the production of many cytokines and chemokines that also have a modulatory effect. Thus, these alterations may have a direct or indirect impact on the release of neurotransmitters, which follows the reduction in spontaneous neuronal activity, and metabolic changes, which are associated with both the viral cycle and neurochemical dysfunction. However, these works do not explain whether these alterations affect only specific neuronal populations, such as GABAergic or glutamatergic neurons, or if they could also be reproduced in nonneural cells (cells from the adaptive and innate immunity). These data may suggest that disease progression involves complex neurochemical changes, affecting energy metabolism, crucial cellular functions, and inhibitory/excitatory neuroimmune homeostasis linked with neuronal failure and host death.

Of note, ACh modulates many aspects of immune signaling. ACh is a crucial regulator of immune functions through both muscarinic and nicotinic receptors expressed by immune cells. Still, many other neuromodulatory molecules, such as catecholamines, peptides, and hormones, regulate the functions of immune cells.<sup>129–131</sup> Many cytokines and immune mediators produced during the infection, such as CXCL-16, may influence neuroglial communication.<sup>64</sup> However, little is known about how the neural changes (e.g., functional changes in the spinal cord and brain) evoked by RABV and other neurotropic agents impact the pathogen-specific responses that would prevent disease outcomes. Furthermore, there is a lack of studies investigating the impact of these infections on the expression profile of neuroimmune receptors in leukocytes to understand the complex, integrative, and systemic interactions in the course of infection. Understanding this neuroimmunological crosstalk in a systemic approach would help to explore new insights into rabies and other neuroinvasive and autoimmune diseases.

## 5 | RABV, THE SYNAPTIC PATHOLOGY IN THE INFECTED BRAIN AND THE GABA-GLUTAMATE HYPOTHESIS

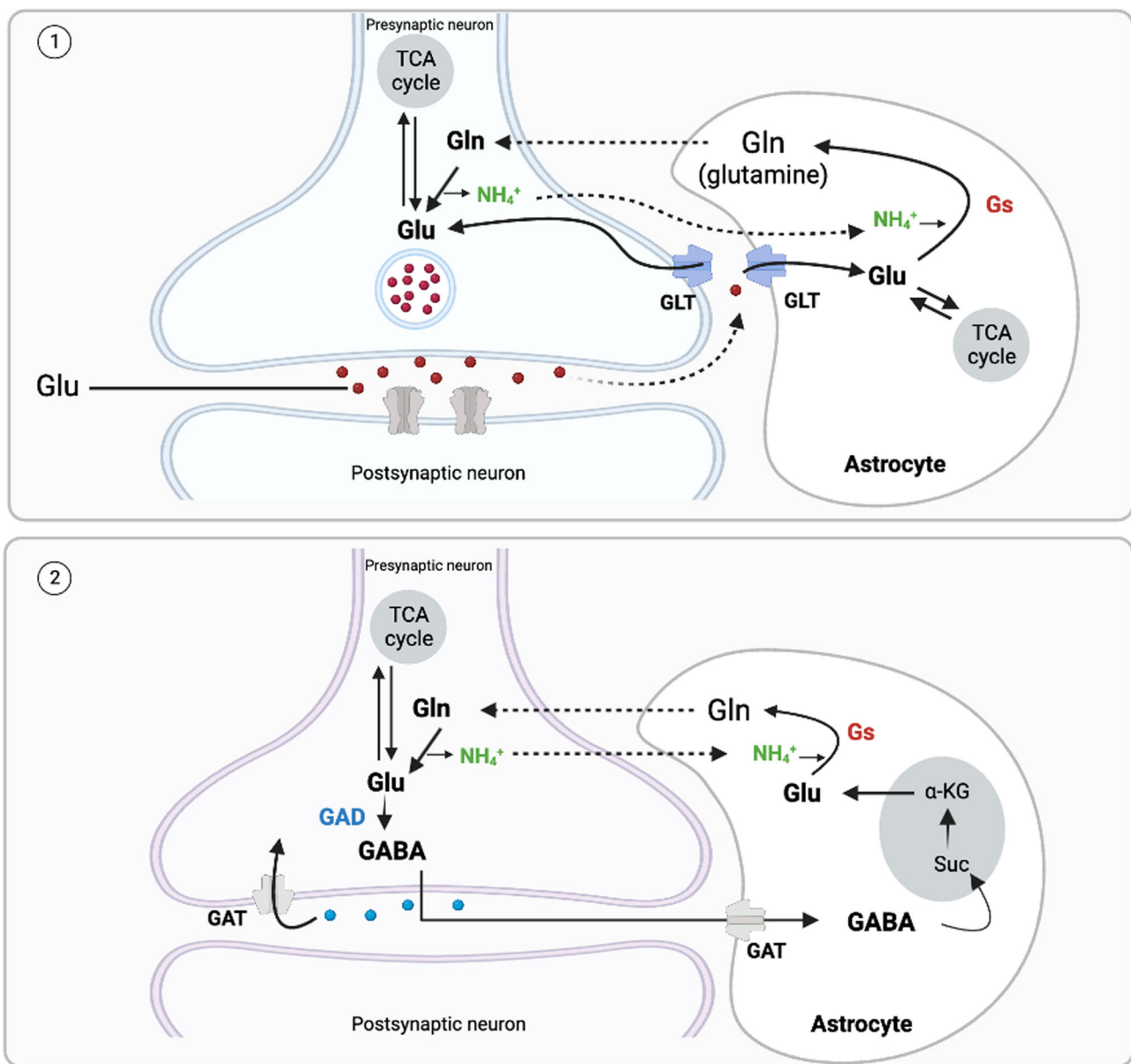
As discussed above, the pathogenesis of rabies in the CNS is complex and diversified according to stage and disease progression. Initially, the infection seems to enhance extracellular levels of neurotransmitters<sup>94</sup> in response to the innate activity of glial cells. However, neurotransmitters' extracellular levels appear to lower as the disease progresses, especially GABA levels.<sup>114</sup> On this topic, we suggest that the dysfunction of the GABAergic machinery may be the primary driver of neuronal pathology since it might affect excitatory/inhibitory homeostasis, which may be one of the leading causes of metabolic and neuronal disorders. We argue that the mechanism by which the CNS may respond to the increased glutamate levels to prevent excitotoxicity should worsen the situation by reducing neuronal activity, contributing to neural failure. This possible microenvironment may also affect the immune system response at the site of infection.

These associations might be necessary because brain homeostasis depends on the balance of functional inhibitory and excitatory signals, represented by GABA and glutamate, respectively. Glutamate, the primary excitatory amino acid neurotransmitter in the NS, regulates a range of physiological functions of the brain and spinal cord under physiological concentrations (5–15  $\mu\text{Mol/g}$ ).<sup>132</sup> Through binding to its metabotropic (mGLURs),<sup>133</sup> and ionotropic receptors, such as the NMDAR,<sup>134</sup>  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA),<sup>135</sup> and kainate receptors,<sup>136</sup> these neurotransmitters modulate a range of neuronal and metabolic functions, playing a crucial role in the viability of neurons. While the excessive glutamatergic signaling during an inflammatory promotes excitotoxicity and calcium-dependent cell death, the depletion of NMDA enhances the apoptosis in developing neurons,<sup>137</sup> turning the “NMDA paradox.”

Thus, sodium-independent and sodium-dependent transport tightly regulates the extracellular glutamate levels. The sodium-dependent glutamate uptake by glial cells is preferentially controlled by excitatory amino acid transporters (EAATs), especially neuroglial EAAT-2 (GLT-1) transporters. GLT-1 transporter is responsible for almost 95% of the glutamatergic uptake and clearance from the extracellular space.<sup>138,139</sup> On the other hand, GABA represents the primary inhibitory neurotransmitter in the CNS. It inhibits the propagation of the action potential and plays a crucial role in regulating excitatory signaling.<sup>140</sup> GABAergic neurons release GABA in the extracellular space upon  $\text{Ca}^{2+}$  influx. Interestingly, these neurotransmitters are metabolically associated since the enzyme glutamic acid decarboxylase (GAD) metabolizes glutamate as the precursor molecule for GABA synthesis (Figure 4).<sup>141</sup>

In summary, GABAergic and glutamatergic neurons differ in their biochemical composition because the first specifically expresses the GAD enzyme,<sup>83</sup> while glutamatergic neurons lack the enzyme. It represents an evolutionary and crucial strategy to prevent excitotoxicity. It should also be mentioned that other neuronal populations can interact with GABAergic and glutamatergic neurons, including the serotonergic system. In rabies, reduced serotonergic neurotransmission has been observed in human<sup>142</sup> and animal models.<sup>143,144</sup> Serotonin was also linked to the aggressive behavior of experimentally infected skunks.<sup>145</sup> However, whether impaired serotonin signaling affects GABA/glutamate release<sup>146,147</sup> or whether serotonergic neurons suffer from impaired GABA/glutamate neurotransmission<sup>148,149</sup> is still unclear.

Astrocytes are the central controller of the metabolisms of both GABA and glutamate because they regulate the uptake and release of those synaptically released neurotransmitters. Once cleared and transported by the EAATs to the intracellular space, astrocytes control the metabolic glutamine-glutamate neurotransmitter cycle. These cells specifically express the glutamine synthetase (GS) enzyme, which converts glutamate to glutamine (Gln). The latter is transported back to neurons for its conversion/hydrolysis to glutamate by glutaminases.<sup>150</sup> In this regard, the astrocytic GABA-glutamine cycle occurs between GABAergic neurons and the surrounding astrocytes. Thus, astrocytes and neurons take up the extracellular GABA by specific GABAergic



**FIGURE 4** Metabolic integration between gamma-aminobutyric acid (GABA) and glutamate. In (1), the synthesis, neurotransmission, and glial glutamate catabolism in glutamatergic neurons. Glutamate may be synthesized from alpha-ketoglutarate ( $\alpha$ -KG) in the tricarboxylic acid cycle (TCA) cycle and glutamine (Gln) by glutaminases. After this release in the synaptic cleft, the neurotransmitter uptake is made mainly by GLT-1 (EAAT2) receptors expressed in the surrounding neuroglial cells for the glial synthesis of glutamine by glutamine synthetase (Gs). The ammonia produced during the conversion of Gln to Glu is used to synthesize glutamine in the astrocytes, which is essential for nitrogen homeostasis in the brain. In (2), metabolism and neurotransmission of GABA in the central nervous system (CNS). GABAergic neurons produce glutamate from the TCA cycle and glutamine, but they specifically express glutamic acid decarboxylase, which converts glutamate to GABA. After the vesicular release of the neurotransmitters, it is captured by neuronal and glial GABA transporters (GAT). Astrocytes control the two-step metabolism of GABA to succinate and then to  $\alpha$ -KG, which is then required to synthesize glutamate and glutamine. Created with [biorender.com](https://biorender.com).

transporters, catabolize it to succinate, and then metabolize it to  $\alpha$ -ketoglutarate ( $\alpha$ -KG). This process is known as the tricarboxylic acid cycle (TCA cycle), which is also required for glutamate synthesis.<sup>151,152</sup> Therefore, glutamate might alternatively be produced by GABA-transaminase (GABA-T) from  $\alpha$ -KG or by GABA-T recycling GABA to succinic acid. It demonstrates the complexity and evolutionary strategy correlating the metabolisms of these two most prevalent regulatory neurotransmitters.<sup>153</sup>

In the particular context of rabies pathogenesis, we have hypothesized that the pro-inflammatory stimuli and GABA dysfunction may enhance the extracellular glutamate levels. This event might be associated with neuropathology and neuronal dysfunction. Although previous works have failed to demonstrate excitotoxicity in vitro,<sup>154</sup> many aspects help to support our hypothesis. Firstly, the metabolic profile of the *post-mortem* brain characterized the intense disturbance of glutamine levels in both the cerebral cortex and white



matter because of the gliosis,<sup>11</sup> and it simultaneously indicated the decrease of GABA levels. So, we argue that the increased glutamine levels might reflect the neurochemical disorder of the glutamatergic pathways in response to the lack of GABA-mediated inhibition upon glutamate levels. Likewise, the quantitative proteomic analysis of the human brain of fatal rabies strengthens this hypothesis by indicating the overexpression of the astrocytic enzyme glutamate ammonia ligase (GLUAL). This removes toxic ammonia produced mainly during glutamate-glutamine metabolism<sup>151</sup> and is directly associated with glutamate signaling in the brain, maintaining nitrogen homeostasis in the CNS.<sup>82</sup>

In addition, glutamate is constitutively released by activated and reactive microglial cells in response to infections.<sup>155</sup> Upon oxidative burst and metabolic changes related to the expression of reactive M1 phenotype during pro-inflammatory stimuli, microglial cells trigger glutamate release through the cystine/glutamate XcT-antiporter-system. It aims to capture cystine from the extracellular space because it is required for the intracellular synthesis of glutathione (GSH), and GSH protects microglia from oxidative stress.<sup>155,156</sup> The substantial levels of glutamate released in response to the GSH depletion trigger neurodegenerative events associated with neuroinflammation, which are attenuated by pathogenic wt RABV strains. In that sense, and during the early transcriptional changes induced by the virus, pathways related to XcT receptor-mediated glutamatergic activity and voltage-gated calcium channels release of glutamate were upregulated,<sup>10</sup> possibly suggesting that it might occur during RABV pathogenesis.

It is also essential to evaluate the protein expression of the EAATs during the viral kinetics because a disturbance in the glial uptake may result in increased glutamate levels.<sup>138</sup> In other neuroinvasive diseases, such as WNV infection, the production of inflammatory cytokines and immune mediators decreased the protein expression of the glial GLT-1. Even though the astrocytic population has become more numerous in response to the infection, the decrease in GLT-1 protein levels led to motor dysfunction.<sup>157</sup> Decreased glial GLT affected the clearance of extracellular glutamate and thus favored chronic cellular damage, resulting in motor impairment. In the context of paralytic rabies, no previous works have traced this association, despite some indications from authors that RABV adsorption induces a transient alteration in the protein expression of presynaptic regulatory receptors.<sup>19</sup> Moreover, evidence from experimental studies suggested that releasing endogenous damage-associated molecular patterns (DAMPs) in the extracellular environment, especially adenosine triphosphate (ATP), enhances the glial production and release of glutamate. It occurs via the purinergic-dependent P2X7 mechanism, leading to a drastic and acute increase in the amounts of the neurotransmitter,<sup>158</sup> which may, in turn, activate the NLRP3 inflammasome<sup>159,160</sup> and help to potentiate the effect.

On the other hand, because of the unique metabolism of the brain, in particular, that of the cortical region, which is directly linked to both GABAergic and glutamatergic synapses, high energy consumption is the direct consequence. However, GABAergic activity

requires less energy catabolism than glutamatergic neurons.<sup>161</sup> Howarth et al.<sup>162</sup> indicated that most energy used for excitatory signaling is related to postsynaptic glutamate NMDA and non-NMDA receptors. They require energy to reverse the influx of  $\text{Ca}^{2+}$  and  $\text{Na}^{2+}$  ions associated with the ionotropic glutamate receptors, showing the influence of excitatory signaling on CNS metabolism homeostasis. In rabies, as previously discussed, the increase in glucose levels and metabolites in the brain may be related to the rise in the system's demand and uptake of glucose to restore homeostasis. We also suggest that these events result from viral replication and excitatory metabolism and are strongly associated with glucose metabolism.<sup>163</sup>

Evaluating how the system responds to that hypothetical scenario would be interesting. Excitotoxicity elicits pro-death stimuli via NMDA and AMPA receptors activation by the overload of calcium and mitochondrial/oxidant stress, resulting in neuronal apoptosis.<sup>164</sup> In addition, the CNS exploits intrinsic and unclear mechanisms to reduce or protect neurons from the toxic effects of over-excitatory stimuli. One of the adaptive mechanisms to protect the brain from excitotoxicity is associated with the redox and toxic control of NMDAR activity.<sup>165</sup> This control is affected by the cellular location and subunit of NMDAR subunits.<sup>166</sup> For instance, Zhu et al.<sup>167</sup> have shown for the first time that dynamic trafficking, upregulation, and control of the GluN2A-NMDA subunit protected cortical neurons from excitatory and oxidative stress. They demonstrated the active role of distinct subpopulations in NMDAR physiology. In that regard, Granzotto<sup>165</sup> recently found that nNOS (+) neurons, which fail to generate reactive oxygen species (ROS) upon NMDA activity, decrease the expression of NMDA GluN1 unit to present more reduced, and thus more functional NMDAR. The authors highlight that this is an intrinsic and cell-autonomous mechanism of the GluN1 subunit to deal with excitatory challenge and balance increased receptor functioning.

In addition, investigating in a holistic and integrative approach how this central (neural) dysfunction (e.g., neurochemical disbalance, oxidative stress, and neuroinflammation) systemically modulates pathogen-specific immunity would help to reveal new pathways of neuroimmune communication, for instance, the role of brain/spinal cord into the modulation of innate and adaptive functions under dyshomeostasis. Since immune cells express adrenergic,<sup>168</sup> cholinergic,<sup>169</sup> glutamatergic, GABA-receptors,<sup>12</sup> and upon cerebral alterations, increased serum levels of neurotransmitters may reflect different physiological states of the nervous system.<sup>170</sup>

Moreover, we have previously shown, the activation of class I mGLURs triggers the migratory capability in neutrophils<sup>24</sup> and modulates T-cell functions through glutamate metabotropic receptors<sup>25</sup> and transporters.<sup>171–173</sup> Therefore, characterizing how this hyperexcited microenvironment affects entering immune cells phenotype would help understand the mechanisms by which viruses evade and indirectly regulate immune functions, as observed during neurodegenerative conditions<sup>174</sup> and rabies.<sup>107</sup>

Finally, we argue that comprehending the temporal dynamics of glutamate receptor control and this metabolic crosstalk under RABV pathology might reveal the neurochemical and neuroimmune basis of



neuroinvasive infections. Considering the complexity of the network interplay between neurotransmitters and their properties in the modulation of immune profiling, future studies aiming to describe the pathophysiological basis of diseases targeting the CNS require integrative systems approaches to comprehend further the role of neurological and hormonal regulation of the immune response.

## 6 | FUTURE PERSPECTIVES

The importance of neuroimmunology studies for health and disease highlights the necessity of characterizing new therapeutic targets and modulators of systemic functions.<sup>175</sup> Besides, paths to understanding rabies pathophysiology and treatment are valuable and have been revealed based on in vitro or ex vivo data. However, they may not reflect what is happening in natura with humans. In this context, it will be essential to understand better how neurotransmitters regulate chemotaxis and cell migration and differentiation of immune cells under health conditions, other infections, and chronic autoimmune diseases, as we have recently performed.<sup>176–180</sup> For instance, integratively understanding the effect of new pathological alterations in rabies and other neuroinvasive infections may bring new answers and perspectives regarding the knowledge of how the micro-environment, host-pathogen interactions, and the nervous system determine pathophysiologic outcomes. In this context, addressing the impact of neuroimmunological alterations on the endocrine system (e.g., hormone levels and pathophysiological functions) will be essential to advance psychoneuroendocrinology mechanisms induced by rabies virus and neuroinvasive agents, which is currently a neglected research field.

Of note, intervention measures must be aimed against viral replication and restoring or rescuing the mitochondria system to gain time and support other organs than the nervous system. Thus, tests to monitor the viability of the brain must be available (such as an animal PET scan to assess neuronal function and functional MRI). Such tools allow the evaluation of any potential in therapeutics to be judged more efficiently.

## 7 | CONCLUSIONS

This review summarized essential findings of RABV neuroimmunology. We present the complexity of host-virus interplay focusing on the neuroimmune, cellular, and neurochemical interactions in response to the infection. Understanding the possible RABV tropism to specific neurobiological systems and the basis of the neurochemical interactions in the context of rabies-associated neuroinflammation would help to explain some contradictions that remain unexplained about the disease pathogenesis and would also help to guide new hypotheses about the basis of the neuroimmune interactions modulation of the anti-RABV immune response. Investigating how the immune system responds to those neurochemical and metabolic disorders may help us characterize novel pharmacological

targets to prevent neuronal dysfunction and cell death in the late stage of RABV pathology.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Research data are not shared.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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