Respiratory viral infections and associated neurological manifestations

Abstract: Respiratory viruses as a major threat to human and animal health today are still a leading cause of worldwide severe pandemics. Although the primary target tissue of these viruses is the lung, they can induce immediate or delayed neuropathological manifestations in humans and animals. Already after the Spanish flu (1918/20) evidence accumulated that neurological diseases can be induced by respiratory viral infections as some patients showed parkinsonism, seizures, or dementia. In the recent outbreak of COVID-19 as well patients suffered from headache, dizziness, nausea, or reduced sense of smell and taste suggesting that SARS-CoV2 may affect the central nervous system (CNS). It was shown that different respiratory viral infections can lead to deleterious complications in the CNS by a direct invasion of the virus into the brain and/or indirect pathways via proinflammatory cytokine expression. Therefore, we will discuss in this review mechanisms how the most prevalent respiratory viruses including influenza and coronaviruses in humans can exert long-lasting detrimental effects on the CNS and possible links to the development of neurodegenerative diseases as an enduring consequence.

Keywords: central nervous system; coronavirus; influenza virus; neurodegeneration; respiratory viral infections.

Background

Respiratory viral infections are one of the most important leading causes of morbidity and mortality particularly among young children, elderly and patients with immunodeficiency, imposing a massive economic burden and critical hazards for public health (Bohmwald et al., 2018). The most important viruses that cause respiratory diseases include coronavirus, influenza virus, human respiratory
syncytial virus (hRSV, *orthopneumoviruses*), human metapneumovirus (hMPV, *metapneumoviruses*) and adenovirus (Desforges et al., 2020; Nichols et al., 2008). Respiratory viral infections usually are self-limiting, involve the upper airways and cause relatively mild symptoms such as sneezing, coughing and nasal congestion. Nevertheless, these mild infections can be dangerous for vulnerable individuals, such as newborns, elderly and immunocompromised persons in that the viruses can impact the lower airways, resulting in shortness of breathing and pneumonia (Troy and Bosco, 2016). Some respiratory viruses are constantly appearing among human populations around the world every year, leading to symptoms ranging from mild to more severe manifestations requiring hospitalization (Desforges et al., 2020). In addition to the seasonal respiratory viruses, new strains emerge in the human population occasionally causing epidemics or even pandemics. These are usually RNA viruses such as influenza A subtypes and human coronavirus strains that are present in an animal reservoir, cross the species barrier selecting a new host. Zoonoses like these can be very dangerous for humans (Berry et al., 2015; Desforges et al., 2020). The latest emerging virus entitled severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) was recently identified in December 2019 in an individual located in Wuhan, China, who had severe symptoms of pneumonia. Subsequently, the world health organization (WHO) named the disease COVID-19 and declared the emergence of a global pandemic with high international concern (Desforges et al., 2020).

It is important to note that besides affecting the respiratory tract, these emerging viruses can have devastating effects also on other parts of the human body, including the central nervous system (CNS) (Table 1), thereby potentially increasing the risk for neurological disorders and neurodegenerative diseases (Bohmwald et al., 2018).

Some respiratory viruses such as neurotropic influenza A strains have the capability to invade the CNS where they can infect neurons and other resident cells and lead to neural dysfunction. However, new findings indicated that also merely peripheral infections caused by respiratory viruses can have substantial impacts on the CNS (Hosseini et al., 2018). Neurodegeneration as well as chronic neuroinflammation induced by respiratory viral infections might moreover trigger pathways involved in classical neurodegenerative disorders in humans. Supporting this, many similarities between prevalent neurodegenerative diseases such as Alzheimer’s and Parkinson’s and virus-mediated neurodegeneration have been identified at the cellular and molecular level (Arbour et al., 2000; Gamboa et al., 1974; Jang et al., 2009). However, the details of how respiratory viral infections affect the CNS are still incompletely understood.

### Neurological manifestations associated with respiratory viral infections

Commonly when referring to virus associated neurological disorders, nonrespiratory viruses which directly invade the CNS such as *Flaviviridae* family members including Japanese encephalitis, Langat or West Nile viruses (Cornelius et al., 2020; Sips et al., 2012), while especially the latter is now a threat in Germany as well (RKI: www.rki.de), are discussed. These neurotropic viruses increase the incidence of dramatic

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<td>Human coronaviruses (SARS-CoV1, MERS-CoV, SARS-CoV2)</td>
<td><em>Coronaviridae</em> family Enveloped viruses with a positive-sense single-stranded RNA genome</td>
<td>Encephalopathy Encephalitis Meningitis Seizures Stroke Neuromuscular disorders Anosmia and dysgeusia Seizures Encephalopathy Encephalitis Confusion Loss of consciousness Transverse myelitis Guillain-Barré syndrome</td>
<td>Alshebri et al. (2020); Ng Kee Kwong et al. (2020); Verstrepen et al. (2020)</td>
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<td>Influenza A viruses (H1N1, H3N2, H7N7, H5N1)</td>
<td><em>Orthomyxoviridae</em> family Enveloped viruses with a negative-sense single-stranded RNA genome</td>
<td></td>
<td>Ekstrand (2012); Khandaker et al. (2012); Mylonaki et al. (2020); Robinson and Busl (2020)</td>
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neurological dysfunction and can even lead to respiratory deficiency induced by neuromuscular impairment (Madden, 2003). In addition, CNS pathology is intensively studied in infections caused by other well-known neurotropic viruses including measles, Varicella-Zoster and human immunodeficiency viruses (HIV) (Koyuncu et al., 2013). However, regarding the increased risk of respiratory viral infections due to spreading in an increasingly globally connected human population, more attention is necessary to also focus research on neurological manifestations induced by these infections (Bohmwald et al., 2018). The most common reported neurological complications associated with severe respiratory infections induced by influenza, coronavirus, hRSV, and hMPV are seizures mostly occurring as febrile seizures with a higher incidence in patients suffering from epilepsy (Bohmwald et al., 2018; Vezzani et al., 2016). Besides this, encephalopathy can also be observed following respiratory infections as well as an expanded spectrum of manifestations including coma with long-term morbidity or mortality and some nonsevere changed mental states accompanied by minimal-to-no sequelae. Occasionally, other neurological disorders can be associated with respiratory infections like stroke, focal neurologic defects, Guillain-Barré syndrome, acute disseminated encephalomyelitis and transverse myelitis (Ekstrand, 2012). Furthermore, respiratory viral infection can induce significant changes in gut microbiota compositions (Groves et al., 2020). A link between the gut microbiota and the brain has long been suggested. Recent findings, however, elucidate the important role that changes in the composition of the gut microbiota play in the development of behavioral disorders such as anxiety and depression (Kim and Shin, 2018).

In line with previous observations, COVID-19 patients show neurological manifestations such as headache, confusion, dizziness, nausea and vomiting as well. In a subset of these patients, stroke, and seizures have also been reported. Furthermore, other unusual neurologic signs such as a diminished sense of smell (anosmia) and taste (dysgeusia) are reported in COVID-19 patients (Paterson et al., 2020). Earlier it was shown that SARS-CoV1 is indeed able to infect the brainstem in both patients and
experimental animals (Netland et al., 2008). Increasing evidence shows that coronaviruses similar to influenza viruses do not always affect only the respiratory system but may also induce neurological consequences either by directly invading the CNS or indirectly by peripheral infection (Figure 1) (Ekstrand, 2012; Li et al., 2020). Nevertheless, in addition to the acute impacts of respiratory viral infections on the CNS, one of the earliest and most important links between respiratory infections and long-term neural dysfunction is a correlation between the 1918 Spanish flu, caused by influenza A H1N1 strain and an epidemic of neurodegenerative disease such as Parkinson’s disease (PD) decades later (Henry et al., 2010). Interestingly, PD-like symptoms such as tremors in the months after seasonal influenza infection have also been reported, however, this is not associated with an increased risk of developing idiopathic PD (Toovey et al., 2011). Moreover, several studies have associated human coronaviruses (HCoV) with multiple sclerosis (MS) in which coronavirus-like particles were detected in brain tissue and cerebrospinal fluid from MS patients (Arbour et al., 2000). One of the major histopathological hallmarks of neurodegenerative diseases are misfolded or aggregated proteins associated with neurotoxicity. However, mechanistic details about the emergence of misfolded protein aggregations already at very early stages of several diseases are still mostly unknown. Recently, it was shown that disrupted lysosomal autophagy and subsequent proteostasis loss induced by respiratory viruses such as influenza and coronaviruses may be an overlooked factor in the onset of protein aggregation such as α-synuclein and β-amyloid (Jang et al., 2009; Marreiros et al., 2020; Sulzer et al., 2020). Therefore, besides acute neurological manifestations, these observations reveal that respiratory viral infections may also induce chronic neurological impairments even triggering or enhancing the development of neurodegenerative disease which represents an important threat for public health.

How can respiratory viral infection reach the CNS?

As a main controlling unit of the human body, the CNS needs to be especially protected from endogenous and exogenous threats (Louveau et al., 2015). Therefore, despite the close connection with the environment, the

Figure 1: Respiratory viruses directly via infection of CNS or indirectly via peripheral infection might have devastating impacts on CNS. [Left panel] Neurotropic respiratory viruses can reach the CNS via direct infection of BCSF and BBB endothelial cells, via a “Trojan horse” (infected monocytes, light blue cell) or entrance via peripheral neural routes such as olfactory sensory neurons and the respiratory tract vagus nerve. The virus can spread in the brain parenchyma and infect the resident cells. This is followed by increased infiltration of peripheral monocytes (light blue cell) and T-cells (green cell) from the periphery and activation of microglia (pink cell) as resident innate immune cells in the brain. Subsequently, the local production or entry of proinflammatory cytokines (i.e., IFN-γ, TNF-α and IL-6; blue circles) from the periphery leads to neuroinflammation as a potential cause of neurological manifestations. [Right panel] Non-neurotropic respiratory viruses cannot enter the CNS, in this case proinflammatory cytokines can, however, enter the CNS (red circles) as well as the infiltration of activated monocytes (light blue cell). This then also leads to the activation of microglia (pink cell) in the brain parenchyma triggering neuroinflammation and neurological consequences.
CNS is largely protected from free entry of noxious molecules, pathogens and circulating immune cells within the blood by the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) of the choroid plexus located in the ventricles of the brain. The integrity of the BBB is maintained by tight junctions between cerebral microvascular endothelium, astrocytic endfeet, pericytes and the extracellular matrix (Engelhardt and Sorokin, 2009; Kaplan et al., 2020). Nevertheless, neurotropic subtypes of respiratory viruses can disrupt the endothelial tight junctions of the BBB and subsequently infect resident cells of the CNS (Figure 1). Furthermore, these viruses can cross the BBB via direct infection of endothelial cells and pericytes using endocytic vesicles in a process called transcytosis. A different way to enter the CNS is the “Trojan horse approach” which allows neurotropic viruses to cross the BBB utilizing infected monocytes or macrophages, also termed hematogenous routes (McGavern and Kang, 2011; Swanson and McGavern, 2015). The BCSFB, unlike the BBB, is located at the epithelial level, in which capillaries are leakier and more permeable to small molecules, thus allowing rapid entry of pathogens and hazardous molecules more easily (Engelhardt and Sorokin, 2009). Some neurotropic viruses can in addition spread to the CNS through peripheral neural routes which can be sensory or motor by retrograde and anterograde neuronal transport using the motor proteins dynein and kinesins. Commonly respiratory viruses use olfactory sensory neurons, facial nerves, the trigeminal ganglion, and especially the respiratory tract vagus nerve as gates to enter the CNS (Figure 1) (Swanson and McGavern, 2015). On the other hand, most of the respiratory viral infections which are known as non-neurotropic are limited to the periphery, commonly to epithelial or endothelial cell surfaces in the airways (Figure 1) (Teijaro et al., 2011). Usually, infection of these cells initiates a cell-autonomous reaction and paracrine signaling from the infected cell to neighboring uninfected cells by secreted cytokines as part of the innate immunity (Koyuncu et al., 2013). Nonetheless, the infection can be resolved by the secretion of infection-specific antibodies and the involvement of T-cells the so-called adaptive immunity.

However, both neurotropic and non-neurotropic viruses may escape the local immune control at the primary site of infection and spread to other tissues. Robust viral replication as well as an overreacting innate immune response causing a “cytokine storm” can lead to severe consequences in patients (Castelli et al., 2020; Teijaro et al., 2011). The cytokine storm was reported following many respiratory viral infections including influenza and coronaviruses (SARS-CoV1 and CoV2) which is the major...
factor underlying acute respiratory distress syndrome (ARDS) induced by these viruses (Tisoncik et al., 2012). A cytokine storm is an aberrant response of the host immune system that induces an exaggerated release of inflammatory mediators. This immunological response includes both proinflammatory (i.e., TNF-α, IFNs, ILs) and anti-inflammatory cytokines (i.e., IL-4, IL-10) as well as chemokines (CXCL10, MCP1, and MIP1α), which are secreted at very high levels in the serum leading to an intense systemic immune reaction. Indeed, the cytokine storm in fatal COVID-19 and other severe respiratory infections is represented by several pathological features such as ARDS and subsequent multiorgan failure (Figure 2). Such a reaction can have a dramatic impact on many organs including the brain, as the BBB and the BCSFB are permeable for most of the cytokines (Castelli et al., 2020).

It has been shown that although the baseline levels of inflammatory cytokines are critical for synaptic plasticity (Hosseini et al., 2020), high levels of them impair neuronal function in the adult brain by their direct effect on neurons or by indirect mechanisms mediated by non-neuronal cells such as brain resident microglia and astrocytes and infiltrating peripheral immune cells (Prieto and Cotman, 2017). Therefore, it can be hypothesized that a cytokine storm can be responsible for neuropathological conditions such as meningitis, encephalitis, meningoencephalitis, resulting even in death (Koyuncu et al., 2013). It is in this respect noteworthy that also neurotropic respiratory viruses can affect the brain in both ways including direct CNS infection as well as cytokine secretion. Therefore, given the fact that both neurotropic and non-neurotropic respiratory viruses can have substantial impacts on the CNS, the detailed mechanisms of neuronal dysfunction caused by two prevalent respiratory viruses in the human population including influenza A viruses and coronaviruses will be outlined here.

**Mechanisms of neural dysfunction induced by respiratory viruses**

**Coronaviruses**

Coronaviruses (CoV) as enveloped positive-stranded RNA viruses belong to the *Coronaviridae* family which usually causes mild-to-moderate common cold symptoms associated with upper airways infection. There are over 100 subtypes of coronaviruses that can circulate among animals and humans. Sometimes these viruses are transmitted from animals to humans in a so-called spill-over process, leading to the development of more severe symptoms. Less than 20 years ago, three new coronaviruses emerged from animal reservoirs and caused severe respiratory diseases and death in humans including SARS-CoV1, MERS-CoV, and the recent SARS-CoV2 which in March 2020 led to the global COVID-19 pandemic. It was shown that indeed several laboratory strains of coronaviruses are able to replicate efficiently in the CNS of mice and other rodents by infiltration via the nasal infection route. Among the infected cell types were particularly microglia and astrocytes, leading to a serious infection especially of the brainstem. It is noteworthy that CoV may first invade peripheral nerve terminals, and then penetrate to the CNS via so-called trans-synaptic transfer (Xu et al., 2005). In animal models, depending on the CoV strain, neurovirulence and pathology vary from mild encephalitis with subsequent clearance of the virus and demyelination to rapidly progressing fatal encephalitis (Perlman and Wheeler, 2016). In addition, a severely impaired motor function was shown following CoV infection in animal models. Interestingly, secretion of TNF-α, IL-6, IL-12, and IL-15 and associated activation of microglia play a critical role in controlling CoV in the CNS.

**Figure 2:** An exaggerated host immune response following severe respiratory viral infection can lead to a cytokine storm with severe consequences. Immune signals upon severe respiratory viral infections such as COVID-19 can induce a positive feedback activation of immune cells resulting in exaggerated release of inflammatory cytokines and chemokines also known as a “cytokine storm”. This is responsible for acute respiratory distress syndrome (ARDS), multiorgan failure and eventually death.
as depletion of these cells resulted in faster viral replication and enhancement of the capacity of CoV to escape adaptive immunity (Li et al., 2004). In humans as well, the neurovirulent properties of some coronavirus subtypes have been confirmed. For instance, the RNA of human coronaviruses was isolated from autopsy samples of multiple sclerosis (MS) and encephalomyelitis patients which points toward a possible persistent infection (Arbour et al., 2000). However, MERS-CoV has never been extracted from neural tissue or fluids in infected humans (Algahtani et al., 2016). So, until now the precise capacity of CoV to penetrate and infect the CNS in humans is not well defined. Nevertheless, several neurological complications such as seizures and four-limb twitching have been reported in SARS-CoV1 and MERS-CoV patients. In addition, computed tomography (CT) observations from MERS-CoV patients indicated signs of intracerebral hemorrhage with significant brain edema and intraventricular space enlargement (Algahtani et al., 2016; Arbour et al., 2000; Bohmwald et al., 2018).

In post mortem brain samples of CoV-infected patients (especially after infection with SARS-CoV1), high levels of in particular proinflammatory IFN-γ induced chemokines such as CXCL9 and CXCL10 were observed. These CXC chemokine family members play a critical role in T cell-mediated immunity in the CNS as they promote the infiltration of CD3+ T lymphocytes in the brain parenchyma by binding to the CXCR3 receptor (Xu et al., 2005). In the brains of patients with Alzheimer’s disease and respective animal models, a high concentration of CXCL10 was found which can be associated with plaque formation and cognitive deficits, suggesting a neuropathogenic role for this chemokine and its receptor (Krauthausen et al., 2015). Moreover, earlier studies revealed a high expression of granulocyte-macrophage colony-stimulating factor (GM-CSF) in the periphery and brain of CoV patients which shows significant activation of myeloid cells invading other organs especially the brain. The high expression of GM-CSF in the brain with known proinflammatory functions plays a pivotal role in the development of autoimmune and inflammatory diseases such as autoimmune encephalomyelitis (Li et al., 2016).

In addition to the previous coronaviruses, in the recent outbreak of novel SARS-CoV2, neurological manifestations such as headache, myalgia, nausea, anosmia, ageusia, psychomotor agitation, encephalopathy, acute cerebrovascular disease and even impaired consciousness have been observed in several patients (Moro et al., 2020; Paterson et al., 2020). Although a large number of case reports with neurological manifestations following COVID-19 have been published, it seems yet too early to determine the precise incidence and prevalence rate of these complications in COVID-19 patients. So far studies indicated various prevalence rates for neurological manifestations in different affected countries. For example, in Wuhan, China approximately 36% of COVID-19 patients showed neurological complications, however, in Europe neurological symptoms were documented in 49% of patients. In a study in the USA only 8% of patients with COVID-19 exhibited neurological sequelae, yet neurological symptoms in 35% of COVID-19 patients were reported in Ankara, Turkey (Moro et al., 2020; Pezzi and Padovani, 2020). At the current state a rather high prevalence of neurological manifestations can be estimated in COVID-19 patients which makes it likely that SARS-CoV2 can penetrate into the CNS. In a recent study, the presence of SARS-CoV2 RNA in the nasopharynx and distinct brain regions was documented postmortem in COVID-19 patients which indeed confirms SARS-CoV2 neurotropism. Apparently, SARS-CoV2 can enter the brain via the neuronal-mucosal interface in the olfactory mucosa and directly spread in the respiratory and cardiovascular control centers located in the medulla oblongata (Meinhardt et al., 2021). Apart from acute neurological consequences of COVID-19, it is still too early to know whether neurodegenerative related manifestations similar to the once reported for instance following the Spanish flu and H5N1 avian flu might indeed occur (Jang et al., 2009). So far only a single case report, of postencephalitic parkinsonism was documented. Specific motor deficits have been documented in some patients as well. Therefore, it has been proposed to particularly monitor COVID-19 patients with long-term hyposmia symptoms for decades to find out the particular role of SARS-CoV2 in the onset and progression of neurodegenerative diseases such Parkinson’s disease (Sulzer et al., 2020).

Mechanistically, the first evidence of the harmful effects of the COVID-19 for brain function came from the fact that the SARS-CoV2 virus similar to SARS-CoV1 utilizes the angiotensin-converting enzyme 2 (ACE2) receptor to invade and infect host cells (Baig et al., 2020). Interestingly, the widely ACE2 expression in different brain areas has been defined (Xia and Lazartigues, 2008). Therefore, it can be concluded that the interaction of SARS-CoV2 with ACE2 receptors expressed in neurons can initiate a neuronal viral replication resulting in neuronal damage and dysfunction and subsequent massive neuroinflammatory processes which even can lead to respiratory failure and death in COVID-19 patients (Baig et al., 2020).
Further laboratory experiments using the neurotropic and non-neurotropic strains of coronaviruses are needed to investigate the virus- and immune-mediated neural dysfunction, subsequent neurodegeneration, demyelination and particularly virus clearance, and/or persistence in the CNS.

**Influenza A viruses**

Influenza A viruses (IAV) as enveloped segmented negative-strand RNA viruses belong to the *Orthomyxoviridae* family which are circulating among animals and the human population. Despite the fact that most individuals recover from an IAV infection, the acute and long-term IAV impacts on the CNS remain mostly elusive.

Some strains of IAV (i.e., H7N7, H7N9, H5N1) are neurovirulent and can spread to the CNS through olfactory, trigeminal, vagus and sympathetic nerves and induce severe neurological manifestations (Park et al., 2002). For instance, the highly pathogenic avian H5N1 IAV can infect the CNS cells including neurons and glial and subsequently lead to neuronal damage and death acutely. Evidence is accumulated that this is induced by neuroinflammation through the activation of brain resident immune cells, microglia, who increase the local proinflammatory cytokine expression and secretion (Jang et al., 2012; Park et al., 2002). In addition, the activation of resident brain immune cells following H5N1 IAV infection leads to a significant increase in phosphorylation and aggregation of alpha-synuclein, resulting in the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and parkinsonian symptoms (Jang et al., 2009). Investigations on other avian neurotropic IAV strains such as H7N9 revealed efficient viral replication, proinflammatory cytokine upregulation and cytopathy in the brain which can induce intensive CNS injury (Ng et al., 2018). Furthermore, in the case of H9N7 neurotropic IAV strain, a significant relationship between infection-induced neuropathology and increased levels of inflammation active factor NLRP3 and related cytokines IL-1β and TNF-α was identified (Yu et al., 2014). In addition to the acute impacts of neurotropic IAV strains on brain cells, we recently investigated the long-term effects of a mouse-adapted neurotropic H7N7 (rSC35M) IAV strain for hippocampal neuron structure and function at time points well beyond the acute phase of infection (30, 60 and 120 days post infection [dpi]) (Hosseini et al., 2018). It is noteworthy that the hippocampus is a highly plastic region in the brain which is involved in learning and memory formation processes (Korte and Schmitz, 2016). Hippocampus is especially vulnerable to neuroinflammation (Lynch, 2002; Vitkovic et al., 2000), and hippocampal neurons show negative structural changes due to the inflammatory processes as a consequence of the virus infection. This can lead to impairments in hippocampal function associated with deficits in learning and memory formation (Atluri et al., 2015). Our own findings revealed significant signs of immune cell infiltration and profound gliosis in the hippocampus of H7N7 infected mice at 30 dpi correlated to deficits in spatial memory formation, impaired long-term potentiation and reduced dendritic spine density in all principal cells of the hippocampus (Hosseini et al., 2018). The H7N7 IAV subtype did not only replicate in the CNS but the infection led to elevated levels of IFN-γ and TNF-α in the hippocampus and a disruption of the BBB. It can be speculated that this pronounced and prolonged inflammatory immune response in the CNS with activation of microglia might be the underlying cause of neuronal damage resulting in cognitive deficits (Figure 3) (Hosseini et al., 2018). Infections with neurotropic viruses have a wide economic burden on society and a wide range of morbidity and mortality worldwide. Therefore, neurotropic influenza viruses are a major challenge to human and animal healthcare systems. This mainly depends on the unique organization of the CNS with different cell types, very sophisticated structures and functions, reduced immune surveillance and limited regeneration capacity (Ludlow et al., 2016).

Interestingly, neurological sequelae induced by influenza infection are not only limited to neurotropic strains but have also been reported following epidemics and pandemics involving non-neurotropic strains, which cause the majority of human infections (i.e., H1N1, H3N2). These viruses are neither neuroinvasive nor neurovirulent and therefore cannot penetrate or replicate in the CNS but only in the respiratory tract (Atluri et al., 2015; Hosseini et al., 2018). Notably, despite this fact, they can acutely induce neurological consequences. For instance, following the influenza A (H1N1) 2009 pandemic neurological manifestations such as seizures were relatively common among infected children (Khankaker et al., 2012). An investigation in mice showed elevated expression of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, a reduction in neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). This was accompanied by an activation of microglial cells upon H1N1 IAV (A/PR/8/34) infection as well as the altered hippocampal structure and learning deficits in mice at seven days post infection (Jurgens et al., 2012). Another study also confirmed that H1N1 IAV (CA/09) infection can induce microglial activation in parallel with a reduction in BDNF and glial cell-derived neurotrophic factor.
levels and an elevation in the immune-modulatory chemokine (C-C motif) ligand 4 (CCL4) expression mainly in the substantia nigra pars compacta (SNpc) and the hippocampus, despite the absence of virus in the brain (Sadasivan et al., 2015).

Nevertheless, long-term impacts of infection with non-neurotropic IAV strains especially on the CNS have not been studied. Therefore, in our recent study, the effects of two different mouse-adapted non-neurotropic IAV strains including H1N1 (PR8) and H3N2 (maHK68) at 30, 60, and 120 dpi for hippocampal neuron structure and function were investigated (Hosseini et al., 2018). Our findings indicate that unlike the short-term effects reported by (Jurgens et al., 2012), H1N1 infection does not lead to long-term impairments in spatial memory formation or neuronal morphology (Figure 3) (Hosseini et al., 2018). Yet, following infection with non-neurotropic H3N2 IAV, immune cell infiltration and signs of gliosis were indeed observed in the hippocampus of infected mice albeit to a lesser extent compared to the neurotropic IAV strain (Hosseini et al., 2018). Moreover, 30 days post H3N2 infection impaired spatial memory formation and long-term potentiation, as well as a reduced spine number, were detected. The H3N2 IAV strain is not able to replicate in the brain, however, a leaky BBB and even locally elevated secretion of TNF-α in the hippocampus and also an increase in the number and activation status of microglia were found (Figure 3) (Hosseini et al., 2018). Although activated microglia are crucial for the host defense against pathogens, prolonged or aberrant activation can have damaging effects on neurons and adversely affect synaptic transmission and structure (Riazi et al., 2015). It was shown previously that activated microglia play a role in synaptic remodeling and plasticity in the infected brain (Vasek et al., 2016).

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<th>Infection dose</th>
<th>Survival rate</th>
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<td>H1N1 (PR8)</td>
<td>Non-neurotropic</td>
<td>Mild</td>
<td>2000 FFU</td>
<td>100%</td>
</tr>
<tr>
<td>H3N2 (maHK68)</td>
<td>Non-neurotropic</td>
<td>Severe</td>
<td>10 FFU</td>
<td>84%</td>
</tr>
<tr>
<td>H7N7 (rS35M)</td>
<td>Neurotropic</td>
<td>Highly severe</td>
<td>10 FFU</td>
<td>76%</td>
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</table>

Figure 3: The destructive effects of influenza A virus (IAV) infection on hippocampal structure and function at 30 dpi. [A] Young adult mice were inoculated with three strains of IAV with different characterizations. [B] Infection with both neurotropic (H7N7) and non-neurotropic (H3N2) IAV subtypes impaired learning ability for a new platform position in the reversal Morris water maze test. [C] Hippocampal slices obtained from H7N7 IAV infected mice exhibited significantly lower induction and maintenance of long-term potentiation (LTP) compared to control, whereas H3N2 IAV infected mice showed reduced maintenance of LTP compared to control. [D] Thirty days following infection with H3N2 and H7N7 IAVs, the spine density of apical dendrites of CA1 neurons decreased. Representative images of Golgi-stained dendritic spines in hippocampal CA1 neurons, scale bar = 2 μm. [E] The activation status of microglia was assessed by counting the number of primary processes. After infection with both H3N2 and H7N7 IAVs, the number of primary processes of microglia in the CA1 hippocampal subregion decreased at 30 dpi. Representative examples of IBA-1 + cells, scale bar = 10 μm [F-G] Levels of TNF-α and IFN-γ were elevated in the hippocampus of IAVs infected mice at 8 dpi. [H] Following injection of Evans blue dye for the assessment of the BBB integrity, at 10 dpi, the dye was well visible macroscopically only in the brain of H7N7-infected mice, whereas in H3N2-infected mice, it was only weakly visible around the ventricle; adapted from Hosseini et al. 2018.
Therefore, the most likely explanation for the destructive effects of non-neurotropic IAV infection on the CNS lies in processes of neuroinflammation particularly caused by the indirect activation of microglia as the local effectors of the brain innate immunity (Barbosa-Silva et al., 2018; Santos et al., 2016).

Nevertheless, in our study, young adult mice showed partial and full recovery at 60 and 120 days, respectively, post H3N2 and H7N7 IAV infection. This investigation provides evidence that neuroinflammation induced by IAV infection can cause longer-lasting, virus-specific alterations in neuronal connectivity that are still detectable one month after infection and are associated with impairments in spatial memory formation. Interestingly, the immune response promoted by IAV infection can be caused even without CNS viral replication (Hosseini et al., 2018).

IAV infection in humans may therefore not only lead to short-term responses in infected organs but may also trigger neuroinflammation and associated chronic alterations in the CNS. As mild-to-moderate cognitive impairments in humans might be compensated in everyday life these consequences may have been overlooked so far. It will thus be important to study whether similar or even more pronounced impairments would be observed in aged individuals as a more vulnerable group. Our recent preliminary findings suggest that the effects of both neurotropic and non-neurotropic IAV strains are significantly more destructive in older individuals than in the young ones. However, the detailed mechanisms of how peripheral inflammatory responses can lead to the activation of microglia and subsequently cognitive deficits need to be examined more closely.

Conclusions

So far, the origin of many neurological disorders, especially neurodegenerative diseases, remains poorly understood. On the other hand, several common human respiratory viruses with or without neuroinvasive capacity can trigger or exacerbate neuropathological manifestations, particularly in vulnerable individuals. Therefore, nowadays respiratory viruses are considered as important agents responsible for CNS pathologies. Influenza viruses (IAV) and coronaviruses (CoV) are key candidates among the respiratory viruses associated with neurological complications (Table 1) (Nichols et al., 2008). A deeper understanding of the underlying mechanistic details of neuroinvasion of respiratory viruses is needed. In addition, the interaction of peripheral immune reactions triggered by these viruses with the CNS (Figure 1) is critical to evaluate potentially pathological short- and long-term consequences.

Our own investigations indicate that influenza infection in young adult mice with neurotropic (H7N7) and even a non-neurotropic (H3N2) virus, can initiate inflammatory cascades via microglia activation in the brain leading to long-term consequences and therefore most likely increases the likelihood to develop neuropsychiatric and neurodegenerative disorders during a life-time. It needs to be emphasized that also the host immune response triggered by the H3N2 IAV subtype in the periphery was able to impair hippocampal function in our mouse model. More strikingly, IAV without direct replication in the brain resulted in prolonged activation of microglia with the detrimental outcomes for cognitive functions (Hosseini et al., 2018). Neuroinflammation in this respect might well be a central mechanism contributing to the generation and progression of a number of neuropsychiatric and neurodegenerative disorders including Alzheimer’s disease, especially in vulnerable individuals. Our unpublished findings confirmed the hypothesis by showing the far more destructive effects of influenza viruses on the hippocampal structure and function in the elderly and genetically Alzheimer’s mouse models. In addition to IAV, several pieces of evidence revealed that coronaviruses with neurotropic properties were isolated from the brain autopsy obtained MS patients and animal mouse models accompanied by neurological manifestations such as motor function deficits. This indicates that some respiratory viruses such as CoV can be added to the growing list of viruses that persist in the CNS. Interestingly, seasonal coronavirus patterns fit the observed occurrence of MS exacerbations in the human population (Arbour et al., 2000). Therefore, by identifying the exact mechanistic details of CNS and peripheral infections following respiratory viruses in the future, novel approaches to modulate the neuroimmune interaction may provide a strategy to prevent deleterious acute and long-term effects of neurotropic and non-neurotropic respiratory viral infections on brain function and integrity, especially in highly susceptible patient groups. Moreover, by investigating the life-time history of a person and the respiratory viral infections they have been exposed to, it might be possible to unravel the mystery of the unknown origin of neurodegenerative diseases and steps can be taken to reduce the risk of their occurrence, for example, via vaccination.
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