Progress in Neurobiology 182 (2019) 101679

Contents lists available at ScienceDirect

Progress in Neurobiology

journal homepage: www.elsevier.com/locate/pneurobio

Perspective article

Opioid antagonists as potential therapeutics for ischemic stroke

Nadia Peyravian[a,b], Emre Dikici[a,b], Sapna Deo[a,b], Michal Toborek[a,b,*], Sylvia Daunert[a,b,c,*]

[a] Department of Biochemistry and Molecular Biology, Miller School of Medicine, University of Miami, USA
[b] Dr. JT Macdonald Foundation Biomedical Nanotechnology Institute of the University of Miami, USA
[c] University of Miami Clinical and Translational Science Institute, USA

ARTICLE INFO

Keywords:
Ischemic stroke
Opioid antagonist
Blood brain barrier
Neuroprotection
Naloxone
Naltrexone

ABSTRACT

Chronic use of prescription opioids exacerbates risk and severity of ischemic stroke. Annually, 6 million people die from stroke worldwide and there are no neuroprotective or neurorestorative agents to improve stroke outcomes and promote recovery. Prescribed opioids such as morphine have been shown to alter tight junction protein expression, resulting in the disruption of the blood brain barrier (BBB), ultimately leading to stroke pathogenesis. Consequently, protection of the BBB has been proposed as a therapeutic strategy for ischemic stroke. This perspective addresses the deficiency in stroke pharmacological options and examines a novel application and repurposing of FDA-approved opioid antagonists as a prospective neuroprotective therapeutic strategy to minimize BBB damage, reduce stroke severity, and promote neural recovery. Future directions discuss potential drug design and delivery methods to enhance these novel therapeutic targets.

1. Introduction

As of 2017, the US government declared the opioid epidemic as a public health emergency that is linked to a number of serious health issues, including an increase in cerebrovascular events such as stroke (Services UDoHaH, 2018a; Update CH., 2018). Chronic prescription opioid use exacerbates risk and severity of ischemic stroke. Simultaneously, stroke is the fifth overall cause of death in the US and costing the US health care system over $30 billion annually (Fluri et al., 2015; Small et al., 2002; Benjamin et al., 2017; Lee et al., 2013; LoCasale et al., 2014). Despite this, treatment options for ischemic stroke remain limited. Currently, there are no FDA-approved treatments for the resulting pathological damage to the blood brain barrier (BBB) that arises from an ischemic stroke and there is a need for novel drugs to promote stroke recovery as there are no approved neuroprotective or neurorestorative treatments for stroke (Sifat et al., 2017). While substantial research for novel treatments for the protection of the brain from damage after a stroke has been conducted in the past decade, success has been limited, and many neuroprotective treatments have failed in safety or efficacy in clinical trials. BBB disruption is a pathological hallmark in ischemic stroke, thus suggesting that protection of the BBB as a therapeutic strategy during stroke and for stroke recovery is of critical importance. Simultaneously, inflammatory responses are activated during ischemic injury. A potential therapeutic strategy is to modulate resulting microglia and macrophage activation in the ischemic region to reduce neuroinflammation and prevent secondary neurodegeneration resulting from phagocytosis of viable neurons. Herein, we survey the current state of stroke recovery interventions centered on neuroprotective agents for stroke recovery, specifically, opioid antagonists. As several reviews focusing on neuroprotection for ischemic stroke have already been published, this paper focuses on using FDA-approved opioid antagonists as novel drug repurposing for promising neuroprotective pharmacological options for ischemic stroke. While the exact mechanism of action of opioid antagonists is not fully understood, this class of drugs provides an attractive therapeutic option for treating ischemic stroke due to their anti-inflammatory properties, reduction of secondary neuronal loss, and minimization of BBB perturbations through suppression of microglial activation and reduction of cytokines, ultimately, proposing a potential recycling of FDA-approved therapeutics for treatment of prescription opioid induced stroke. An examination and critical review of promising work involving the use of opioid antagonists as prospective stroke therapeutics, and their respective efficacy in primitive human studies and later animal models is discussed.

* Corresponding authors at: Department of Biochemistry and Molecular Biology, Miller School of Medicine, University of Miami, 1011 NW 15th Street, Miami, FL, 33136, USA.

E-mail addresses: mtoborek@med.miami.edu (M. Toborek), SDaunert@med.miami.edu (S. Daunert).

https://doi.org/10.1016/j.pneurobio.2019.101679
Received 7 May 2019; Received in revised form 1 July 2019; Accepted 31 July 2019
Available online 06 August 2019
0301-0082/

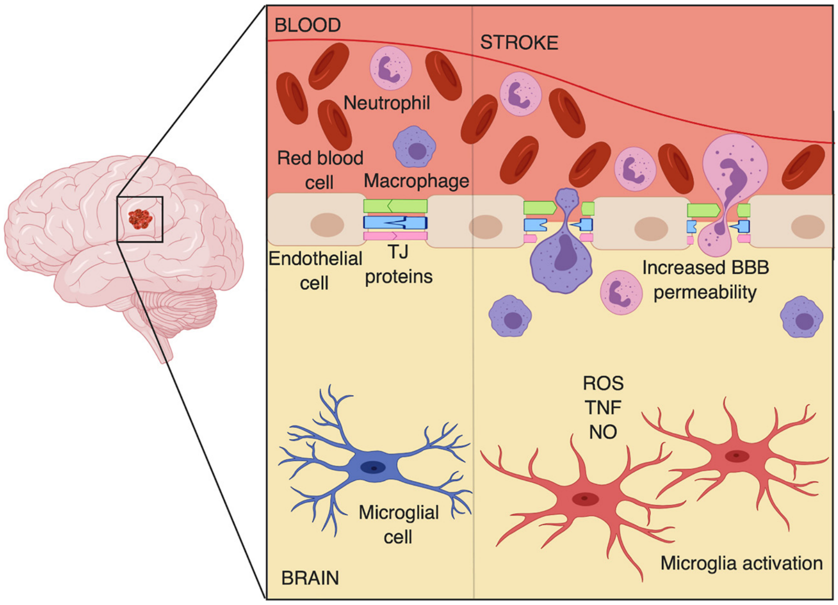

Fig. 1. Blood-brain barrier (BBB) disruption during ischemic stroke. Ischemia, caused by restricted blood flow, results in activation of microglia, leading to release of reactive oxidative species (ROS), nitric oxide (NO), and inflammatory cytokines, such as TNF-alpha, in turn compromising the integrity of BBB. Tight junction (TJ) proteins, such as occludin, junctional adhesion molecule (JAM), and zonula occludens (ZO), become also disrupted, further contributing to dysfunction of the BBB. Dysregulation of TJ proteins results in increased BBB permeability and entry of blood-borne substances and cells, such as macrophages and neutrophils, into the infarct zone and brain parenchyma.

2. Background

2.1. Ischemic stroke

Stroke is the 5th leading causes of death in US, and attributes to 1 of every 20 deaths (Yang et al., 2017; Rodgers, 2013). An ischemic stroke accounts for 87% of all strokes and occurs when there is an obstruction in the blood vessel, such as a blood clot, and fresh blood can no longer reach the brain (Benjamin et al., 2017). When a blockage occurs, the brain lacks the oxygen and nutrients needed for cellular energy, resulting in necrosis (Ferdinand and Roffe, 2016). During an ischemic stroke, the BBB is disrupted (Obermeier et al., 2013; Khatri et al., 2012; Bertrand et al., 2019, 2016).

In ischemic stroke, intracellular tight junctions (TJs) are disrupted, resulting in compromised BBB integrity and increased permeability and poor regulation of transfer of molecules and ions across the BBB (Fig. 1). Often, when BBB integrity is disturbed, neuronal dysfunction, neuroinflammation, and neurodegeneration may occur (Obermeier et al., 2013; Disdier et al., 2017; Carvey et al., 2009). During an ischemic stroke, the affected area suffers oxidative stress, in turn challenging the integrity of the BBB and resulting in breakdown (Fig. 1) (Shirley et al., 2014; Rodrigo et al., 2013; Allen and Bayraktutan, 2009). Oxidative stress is indicative of an increase in reactive oxygen species (ROS) which aids in TJ protein dysregulation (Rodrigo et al., 2013; Rao, 2008; Schreibelt et al., 2007). Much of the vascular and tissue damage in stroke is attributed to neuroinflammation and oxidative stress, and oxidative stress may be one of the underlying mechanisms of BBB disruption in ischemic stroke (Obermeier et al., 2013; Bertrand et al., 2019, 2016; Luissint et al., 2012; Lehner et al., 2011).

Down-regulation or dysregulation of TJ proteins such as occludin and claudin-5 is frequently observed in ischemic stroke (Luissint et al., 2012; Cummins, 2012). TJ proteins such as occludin, junctional adhesion molecule (JAM), and submembranous zonula occludens (ZO) proteins are crucial to the cytoskeleton of the BBB as they regulate cellular traffic into the central nervous system (CNS) (Luissint et al., 2012; Cummins, 2012; Balbuena et al., 2011; Stamatovic et al., 2008). Dysregulation of these proteins can promote the migration of inflammatory cells across the BBB, resulting in neuroinflammation. BBB dysfunction following ischemic stroke has been suggested to be progressive or biphasic, and the time-course of the post stroke BBB opening is not clearly understood (Hone et al., 2018). Several studies have reported opposing data indicating that is unclear if post stroke damage may occur progressively following the stroke or if BBB disruption is exceptional during the first 3 h after stroke, or BBB permeability is biphasic such that significant damage is observed at 4–6 h and then again at 24 or 72 h following a stroke (Giraud et al., 2015; Hjort et al., 2008; Kuntz et al., 2014; Chen et al., 2018). Disruption to the BBB results in increased barrier permeability to blood-borne substances, including leakage of blood proteins (*i.e.*, albumin) as well as monocytes and neutrophils into the CNS, ultimately challenging the homeostasis of the brain microenvironment that is necessary for proper neural functioning (Lulit Price and Grant, 2016; Patel and Frey, 2015; Wunder et al., 2012). This sequence of events has been observed in numerous clinical studies and confirmed in experimental models of a widely used rodent model of ischemic stroke, transient middle cerebral artery occlusion (MCAO) (Fluri et al., 2015; Bertrand et al., 2019; Chiang et al., 2011; Saunders et al., 2015).

2.2. Opioids and stroke

Pain management is critical in the effective care of patients after surgery, as well as patients with cancer, and severe acute and chronic diseases (Russell K Portenoy et al., 2018; Fallon et al., 2018). For example, opioids have been a basis of cancer pain treatment regimen, and morphine and its derivatives are the most used opioid drugs (Nersesyan and Slavin, 2007; Wiffen et al., 2016; Simmons et al., 2012). The action of these opioids is mediated primarily through activation of the μ-opioid receptor. As the principle target for opioids, the μ-opioid receptor is a G protein coupled receptor (GPCR) on brain endothelial cells with high affinity and specific binding towards commonly clinically used opioids such as morphine. While the molecular basis of the μ-opioid receptors is not clearly understood, the μ-opioid receptor mediates the effects of morphine through activation of downstream G-proteins and stimulation of various signaling pathways such as mitogen-activated protein kinase (MAPK)-pathway (Feng et al., 2012; Yang et al., 2011; Chaves et al., 2017; Leo et al., 2009). Morphine is the ultimate analgesic, but, unfortunately, is also highly addictive (Simmons et al., 2012; Morgan and Christie, 2011; Woller et al., 2012). Long-term pain management with opioids present severe side effects, including addiction, abuse, and neurovascular complications, such as ischemic stroke (Woller et al., 2012; Fields, 2011; Rosenblum et al., 2008). Chronic use of prescription opioids induces mitochondrial dysfunction and oxidative stress, which are critical factors in stimulating neuroimmune activation. As a result, these painkillers are now linked to

Table 1
Therapeutic agents undergoing clinical trials for stroke treatment. Updated from Small et al. (2002).

Drug Class	Drug
Drugs for improving blood flow	
Antithrombotic	Heparin, Nadroparin, Tinzaparin, Danaparoid
Anti-platelet	Aspirin, Abciximab
Fibrinogen depleting	Ancrod
Improve capillary flow	Pentoxifylline
Thrombolytics	Pro-urokinase, Tissue plasminogen activator, Streptokinase, Urokinase
Drugs to protect brain tissue (neuroprotective agents)	
Calcium channel blockers	Nimodipine, Flunarizine
Free radical scavengers-antioxidants	Ebselen, Tirilazad, NYP-059
GABA agonists	Clomethiazole
AMPA antagonists	GYKI 52466, NBQX, YM90K, YM872, ZK-200775 (MPQX)
Kainate antagonist	SYM 2081
Competitive NMDA antagonists	CGS 19755 (Selfotel)
NMDA channel blockers	Aptiganel (Cerestat), Dextrorphan, Dextromethorphan
Magnesium	Memantine, MK-801, NPS 1506, AR-R15896AR, HU-211, Remacemide
Glycine site antagonists	ACEA 1021, GV 150526
Polyamine site antagonists	Eliprodil, Ifenprodil
Growth factors	Fibroblast Growth factor (bFGF)
Leukocyte adhesion inhibitor	Anti-ICAM antibody (Enlimonab), Hu23F2G
Nitric oxide inhibitor	Lubeluzole
Opioid antagonists	Naloxone, Nalmefene
Phosphatidylcholine precursor	Citicoline (CDP-choline)
Serotonin agonists	Bay x 3072
Sodium channel blockers	Fosphenytoin, Lubeluzole, 619C89
Potassium channel opener	BMS-204352

higher risk for stroke by compressing the carotid artery or causing cardio-embolism, hypoxia, or hypoperfusion (Lee et al., 2013; Hamzei Moqaddam et al., 2009; Hamzei-Moghaddam et al., 2013; Yu YP, 2016; Baldini et al., 2012). Pathologically, chronic opioid use is also shown to alter the BBB integrity (Baba et al., 1988; Mahajan et al., 2008). Morphine contributes to the breakdown of BBB by disrupting the expression of TJ proteins (Mahajan et al., 2008). Exposure to morphine results in a significant increase in the transendothelial migration of peripheral blood mononuclear cells (PBMC). In addition, increased JAM-2 expression, decreased ZO-1 and occludin gene expression are observed, thus compromising the integrity of the BBB (Mahajan et al., 2008). For example, prostate cancer patients receiving intense morphine had approximately a 3-fold higher risk for ischemic stroke in comparison to non-morphine users. This risk was found to also be enhanced with increased morphine dosage (Lee et al., 2013).

In addition to opioids, opium has been linked to stroke in several clinical studies (Hamzei Moqaddam et al., 2009; Hamzei-Moghaddam et al., 2013; Ebrahimi et al., 2018). Nearly half of a cohort of 35 ischemic stroke consisting of 14 men and 21 women that expressed comorbidity with muscle weakness were observed to have suffered from opium abuse. Consequently, opium abuse was the most common risk factor for ischemic stroke in this study (Iranmanesh, 2008). Similarly, nearly 40% of a sample of 97 ischemic stroke patients that also experienced large vessel involvement such as a large artery stenosis, were found to be dependent on opium (Hamzei-Moghaddam et al., 2013). The relationship between stroke and opium dependence was also studied in a case-control study of 105 stroke and 105 control patients (Hamzei Moqaddam et al., 2009). Patients were diagnosed with stroke by clinical diagnosis and CT scan and opium dependency was confirmed by patients' medical history and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) diagnosis. Analysis of the results indicated statistical significance, therefore opium dependency was suggested as a plausible independent risk factor for stroke (Hamzei Moqaddam et al., 2009).

Opioids have also been linked to an increased prevalence of atrial fibrillation, which is a significant risk factor for stroke (Qureshi et al., 2015; Allouche et al., 2014; Wolf and Kannel, 1991). The prevalence of atrial fibrillation has been observed to be significantly higher in hydrocodone, propoxyphene, and tramadol users in comparison to non-opioid users (12.5% vs 7.6%; $p < 0.001$) in a cross-sectional association between prescription opioid use and atrial fibrillation using data collected from 30,239 participants (Qureshi et al., 2015). This association between opioids and atrial fibrillation may be explained by the down-regulation of opioid receptors (Qureshi et al., 2015). As chronic opioid use leads to tolerance, a decrease in opioid receptor signaling is observed, indicative of an opioid receptor desensitization (Allouche et al., 2014; Rivat and Ballantyne, 2016). This mechanism was proposed in rats that were chronically exposed to morphine and μ-opioid receptor (MOR) activity was reduced compared to animals that did not receive morphine (Allouche et al., 2014). Conventionally, during ischemia, endogenous opioids can exhibit cardioprotective effects by opening mitochondrial K^+ ATP channels, as a protective mechanism against oxidative stress. However, this protective mechanism may be lost with chronic opioid use, causing damage to atrial myocytes and eventually leading to atrial fibrillation (Qureshi et al., 2015).

2.3. Current stroke treatment

Recent advances have been made in preventing the occurrence of stroke, however there are only few therapeutic agents for treatment of ischemic stroke. Currently, there is only one FDA approved drug for stroke treatment: tissue-type plasminogen activator (tPA) (Fluri et al., 2015; Bansal et al., 2013). Recombinant tPA (r-tPA) is a thrombolytic protein that was approved in 1996 as an acute stroke treatment to dissolve the blood clot and restore blood flow to the brain (Sifat et al., 2017; Liu et al., 2018). However, there are many limitation to this drug including a narrow therapeutic window, thus the patient must receive tPA between 3–4.5 h after their stroke onset (Sifat et al., 2017; Liu et al., 2018; Gladstone and Black, 2001; Dela Pena et al., 2018). As less than 15% of patients arrive to the hospital within this window, and, in addition, patients with certain medical conditions are excluded from receiving tPA, only 3% of ischemic patients are eligible to receive this treatment (Small et al., 2002; Sifat et al., 2017). An impaired BBB, such as that exhibited in stroke, also limits the uses of tPA by increasing likelihood of a hemorrhagic transformation (HT) (Lakhan et al., 2013; Tan et al., 2015). Further, tPA has no apparent neuroprotective or neurological recovery effects.

Unlike tPA that targets the thrombus, neuroprotective agents are potential stroke therapeutics that aim to minimize BBB damage and secondary neural damage before and after ischemic injury. Neuroprotective treatments intend to restore or reverse the injury that has occurred to the ischemic region, subsequently to prevent greater or irreversible injury to the ischemic brain (Table 1) (Lutsep and Clark, 2001). Next, we will draw attention to FDA-approved opioid antagonists and their novel use as prospective neuroprotective agents for stroke.

3. Novel therapeutic strategies for ischemic stroke

Various therapeutic agents are being tested in clinical trials for stroke including antithrombotics, antiplatelet agents, and thrombolytics (Table 1). Nonetheless, their uses are limited to dissolving the blood clot and restoring blood flow (Small et al., 2002). As protection of the BBB has been suggested as a therapeutic strategy for ischemic stroke, we surveyed neuroprotective agents for stroke recovery, specifically opioid antagonists, as other neuroprotective agents such as N-methyl-D-aspartate (NMDA) antagonists and gamma-aminobutyric acid (GABA), agonists have been previously extensively reviewed (Table 1) (Small et al., 2002; Sifat et al., 2017; Baba et al., 1988). Protection of the BBB

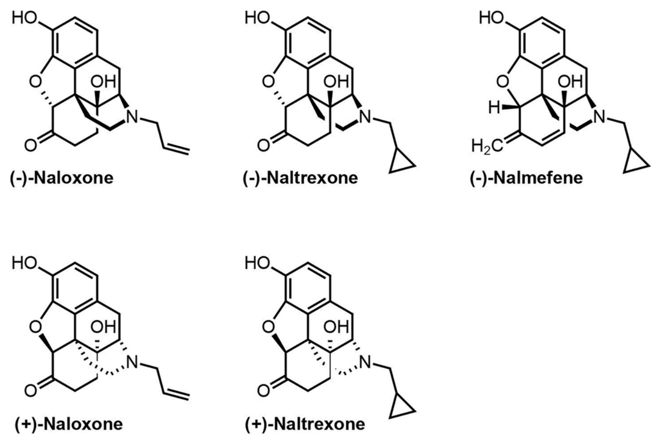

Fig. 2. Chemical structures of surveyed opioid antagonists.

should be prioritized during a stroke and developed as a therapeutic tool for stroke recovery (Sifat et al., 2017; Abdullahi et al., 2018). Combining therapeutic agents with tPA can help to minimize BBB perturbations and appears to be an attractive therapeutic objective (Gravanis and Tsirka, 2008). For example, a study based on an opioid use in a mouse model observed that a small dose of an opioid antagonist, naloxone, significantly reduced the effects of morphine on BBB permeability, suggesting that naloxone may have neuroprotective effects (Baba et al., 1988). Novel therapeutic agents, in conjunction with tPA, that are aimed to minimize BBB perturbation may also minimize the risk for hemorrhagic transformation and increase the therapeutic window of tPA, in turn, increasing the applicability of the drug for a larger number of stroke patients (Sifat et al., 2017).

3.1. Naloxone as a potential therapeutic for ischemic stroke

Naloxone ((-)-naloxone) is an FDA-approved opioid overdose treatment and is administered as a nasal spray or injection (Services UDoHaH, 2018b; Lewis et al., 2017; Ryan and Dunne, 2018) (Fig. 2). It functions as a competitive antagonist by quickly occupying opioid receptors, preventing opiates from binding and activating the receptors (Helm et al., 2008). Initial dosing is one spray (0.4 mg/mL) intranasally or an injection of 0.4 mg/mL for opioid overdose (Rzasa Lynn and Galinkin, 2018; Reference PD, 2019). Although commonly used as opioid abuse medication, naloxone treatment has also been proposed as a promising treatment for ischemic stroke. Naloxone was first suggested as a therapeutic agent for cerebral ischemia in 1981, and its respective neuroprotective effects was initially observed in humans (Baskin and Hosobuchi, 1981). In an initial study, repeated intravenous naloxone was concluded to reverse secondary cerebral ischemia neurological deficits, such as hemiplegia in two human patients (Baskin and Hosobuchi, 1981). In another clinical study, the potential neurorestorative effects of naloxone was observed in thirteen patients with acute stroke that presented neurologic deficits. More than half of these patients returned to their pre-stroke neurological state by the end of their hospital stay after intravenous administration of naloxone (Jabaily and Davis, 1984). Naloxone was also shown to reduce neurologic deficits in opioid use animal models of ischemic stroke, specifically MCAO ischemic stroke in gerbils that received morphine sulfate (Hosobuchi et al., 1982). Intraperitoneal injection of naloxone at 1 mg/kg was found to reverse signs of stroke within minutes of administration, albeit the effect lasted for only 30 min (Hosobuchi et al., 1982). While limited to a small sample size and/or experimental stroke models, these primitive human and animal studies indicate that naloxone administration may be an effective neurorestorative therapeutic to reverse neurologic deficits in acute stroke models (Baskin and Hosobuchi, 1981; Hosobuchi et al., 1982) (Table 2).

The neuroprotective mechanisms of naloxone are not clearly understood (Fig. 3). While many studies suggest that this neuroprotection occurs via blocking opioid receptor activation, other reports have shown that the neuroprotective effects are independent of opioid receptors (Fig. 3). In a study observing the neuroprotective impact of naloxone against ischemic injury in rats, blockage of opioid receptor activation was suggested as a method for decreasing extent of ischemic injury (Liao et al., 2003). To test if opioid receptors are involved in the neuroprotective role of naloxone, (-)-naloxone was compared to its enantiomer, (+)-naloxone, an inactive form of the drug that is not a competitive antagonist for opiates and binding to opioid receptors (Fig. 2). Results found that intracerebroventricular infusion of (-)-naloxone significantly reduced the extent of infarct volume in comparison to enantiomer (+)-naloxone that was ineffective (Liao et al., 2003). As a result, naloxone's neuroprotective role was concluded to involve an opioid receptor mechanism via blocking μ-opioid receptor properties (Fig. 3A) (Table 2). Naloxone was also found to significantly decrease inflammatory cell accumulation as quantified by myeloperoxidase (MPO) activity. Blocking μ-opioid receptor activation by an opioid antagonist was observed to be protective against ischemic injury as brain infarction and neutrophil accumulation were conclusively reduced with naloxone treatment in rat models of ischemic injury (Fig. 3A) (Liao et al., 2003). Similarly, treatment with (-)-naloxone (1 mg/mL or 10 mg/mL) prior to cerebral ischemic injury significantly reduced the extent of the ischemic brain injury in MCAO rats (Chen et al., 2001). Accumulation of inflammatory cells such as neutrophils, macrophages, leukocytes, and microglia is also a hallmark of ischemic injury as a consequence of compromised BBB integrity and increased barrier permeability (Fig. 1). Simultaneously, as MPO activity in the ischemic area is increased within 24 h of injury, pre-treatment with naloxone was found to attenuate this event. These findings not only suggest that naloxone may reduce ischemic neuronal loss and cell infiltration by reducing microglia activation in rats with ischemic brain injury, but also qualify naloxone as a promising effective neuroprotective agent for reducing ischemic injuries (Chen et al., 2001) (Table 2).

Microgliosis occurs as a response to ischemia and results in a neurotoxic environment (Grace et al., 2015). During an ischemic injury, stressed cells release danger-associated molecular pattern molecules that are agonists for Toll-like receptor 4 (TLR4) which induces microgliosis (Fig. 3B). As a result, neurotoxic mediators such as TNFα and IL-1β are released (Grace et al., 2015). Naloxone's anti-inflammatory properties and its respective suppression of microglial activation were studied in the MCAO rat model (Anttila et al., 2018). Activation of microglia was the most pronounced on day 7 post-ischemic stroke and neuronal loss was observed in the thalamus 14 days after MCAO. (-)-Naloxone and its enantiomer, (+)-naloxone, were synthesized and intranasally administered, evaluating if difference in affinity to opioid receptor antagonist by naloxone isoforms may result in varying neuroprotective effects and behavioral recovery. One day after MCAO, (+)-naloxone was administered to rats at a dose of 0.32 mg/kg every 12h for 7 days. On days 10 and 14, body asymmetry and neurological deficits were all significantly reduced in the ischemic rats. By day 14, measured locomotor activity was significantly improved. (+)-Naloxone (0.32–0.8 mg/kg) administered post-stroke also significantly reduced infarction size on day 14 post-stroke and prevented delayed neuronal death. (–)-Naloxone (0.32 mg/kg, administered intranasally) was shown to reduce body asymmetry on days 10 and 14 following stroke. Findings from this study indicated that post-stroke intranasal administration of naloxone to MCAO rat models of ischemic stroke reduces neuroinflammation and promotes behavioral recovery, suggesting that targeting microglia/macrophage activation within the regions of ischemia may be a potential target for stroke therapeutic agents (Anttila et al., 2018) (Table 2). Therefore, it is also suggested that the efficacy of (+)-naloxone in reducing stroke symptoms and its' respective anti-inflammatory and neuroprotective effects may be independent of opioid

Table 2
Survey of opioid antagonists for promoting stroke recovery.

Opioid antagonist	Dose	Frequency	Organism	Disease	Reference
Naloxone					
(–)-Naloxone	0.4 mg intravenous injection	Repeated as needed	Humans	Ischemic stroke	Jabaily and Davis (1984)
(–)-Naloxone	1 mg/kg intraperitoneal injection	Repeated as needed	Gerbils	MCAO ischemic stroke	Hosobuchi et al. (1982)
(–)-Naloxone	0. 4 mg–1.2 mg intravenous injection	2-3 doses	Humans	Ischemic stroke	Reference PD (2019)
(–)-Naloxone	82.5 nmol intracerebroventricular infusion (i.c.v)	Every 4 h	Rats	MCAO ischemic stroke	Liao et al. (2003)
(–)-Naloxone	1 mg/mL or 10 mg/mL intracerebroventricular infusion (i.c.v)	Every 4 h	Rats	MCAO ischemic stroke	Chen et al. (2001)
(-)-Naloxone	0.32 mg/kg intranasally	Twice a day for 7 days	Rats	MCAO ischemic stroke	Anttila et al., 2018
(–)-Naloxone	10 mg/kg initial intraperitoneal injection, 5 mg/kg/h subcutaneously	Continuous	Feline	MCAO ischemic stroke	Baskin et al. (1985)
Naloxone enantiomer					
(+)-Naloxone	0.32 mg/kg - 0.8 mg/kg intranasally	Twice a day for 7 days	Rats	MCAO ischemic stroke	Anttila et al. (2018)
Naltrexone					
(-)-Naltrexone	10 mg/kg initial intraperitoneal injection, 1 mg/kg/h subcutaneously	Continuous	Feline	MCAO ischemic stroke	Baskin et al. (1985)
Naltrexone enantiomer					
(+)-Naltrexone	3 mg/kg or 6 mg/kg intraperitoneal injection	Twice a day for 2 days	Mouse	Cardiac arrest	Grace et al. (2015)
Nalmefene					
Nalmefene	0.05 mg/kg initial dose intravenously, then 0.01 mg/kg	24 h	Humans	Ischemic stroke	Clark et al. (1996)
Nalmefene	0.2 mg intravenous injection	Twice a day for 10 days	Humans	Large cerebral infarction	Li and Song (2017)

receptors.

3.2. Naltrexone as a potential therapeutic for ischemic stroke

As ischemic injury leads to microglia and macrophage activation, which in turn results in neuroinflammation and neuronal loss, the neuroprotective role of naltrexone has been considered. (–)-Naltrexone is an FDA-approved opioid antagonist for opioid addiction that may also be neuroprotective following an ischemic injury (Grace et al., 2015; Goonoo et al., 2014; Liu et al., 2014) (Fig. 2; Table 2). The neuroprotective capacity of (+)-naltrexone, an enantiomer of naltrexone, was observed in reducing microgliosis, neuronal injury, and neuronal death after cardiac arrest (CA) and cardiopulmonary resuscitation (CPR) in mice (Grace et al., 2015) (Table 2). CA was induced in mice by injecting cold KCl into the jugular catheter, and confirmed by EKG. CPR was given after 8 min of CA by epinephrine injection, chest compressions and oxygen ventilation. CA/CPR leads to microglial activation and therefore an increase in pro-inflammatory cytokines such as TNF and IL-1β is observed. (+)-Naltrexone intraperitoneal injection was administered at either 3 mg/kg or 6 mg/kg doses to mice twice a day for two days 30 min after CA. (+)-Naltrexone was used in place of its stereoisomer (–)-naltrexone as it blocks TLR4 signaling and does not bind opioid receptors. Both doses of (+)-naltrexone were shown to significantly protect against ischemic cell death, while the 6 mg/kg dose showed greater neuron protection (Table 2). (+)-Naltrexone was also observed to significantly attenuate production of inflammatory cytokines by microglia and lymphocyte cell infiltration in the mice which is common during BBB disruption. Conclusively, (+)-naltrexone was suggested to be beneficial for reducing neuronal death and neurotoxicity by blocking TLR4 activation (Fig. 3B) (Grace et al., 2015).

Acute and long-term effects of continuous naloxone and naltrexone administration were shown to improve motor function after an ischemic stroke in a feline model of cerebral ischemia generated using MCAO (Baskin et al., 1985). Naloxone or naltrexone intraperitoneal injection, both administered at an initial dose of 10 mg/kg and then transferred to a lower continuous dose for 24 h, significantly improved motor function and prolonged survival of cats with MCAO compared to controls receiving the saline (control) injection. Moreover, a significant improvement in motor function was observed with naloxone and naltrexone administration, and cats regained normal walking abilities. These results suggest that naloxone and naltrexone opiate antagonists may have neurorestorative neurologic effects and may be useful in treating ischemic neurologic deficits (Baskin et al., 1985) (Table 2).

Conclusively, the studies above highlight the e potential neuroprotective and neurorestorative properties of opioid receptor antagonists, naloxone and naltrexone (Table 2). As previously stated however, the mechanisms by which these antagonists elicit their effects are not fully understood. Additionally, naloxone and naltrexone have been suggested to inhibit NADPH (dihydronicotinamide adenine dinucleotide phosphate) oxidase (NOX2), an enzyme complex responsible for oxidative stress (Fig. 3C) (Younger et al., 2014; Wang et al., 2012). Above, we described the increase in oxidative stress as a result of microglial activation in the pathogenesis of ischemic stroke and comprised BBB integrity. In order to inhibit the increase in oxidative stress and stroke progression, blockage of NADPH oxidase (or NOX2) has been suggested (Qin et al., 2005). This enzyme complex consists of membrane bound $gp91^{phox}$ subunit and $p22^{phox}$, as well as three cytosolic proteins ($p40^{phox}$, $p47^{phox}$, and $p67^{phox}$). Upon cell activation, these cytosolic components are translocated to plasma membrane to interact with the membrane bound $gp91^{phox}$ subunit and $p22^{phox}$ to assemble an active NADPH oxidase enzyme complex resulting in superoxide O_2– generation (Fig. 3C). Naloxone and naltrexone may function by inhibiting enzymatic activity of NADPH oxidase by binding to the $gp91^{phox}$ subunit and inducing a conformational change of the NADPH protein complex, affecting the binding affinity of the cytosolic subunits, $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$. Consequently, pro-inflammatory cytokine production, ROS, and NO that compromise BBB integrity are reduced as suggested by *in vitro* studies (Lehner et al., 2011; Wang et al., 2012). Naloxone inhibition of superoxide production is suggested to be independent of opioid receptors as superoxide production induced by LPS (lipopolysaccharide) was significantly and dose-dependently inhibited by (–) and (+)-naloxone isomers (78). Direct targeting of NOX2 and suppression of superoxide generation by naloxone was studied using blood neutrophils due to their abundance of NOX2. Neutrophils were treated with PMA (phorbol myristate acetate), a commonly used agent for superoxide production to stimulate NOX2. Naloxone was found to inhibit NADPH-dependent superoxide generation by PMA-stimulated neutrophil membranes, indicating a direct inhibitory effect of naloxone on NOX2 (Fig. 3C) (Baskin and Hosobuchi, 1981).

3.3. Nalmefene as a potential therapeutic for ischemic stroke

Nalmefene is an opioid receptor antagonist that has also been

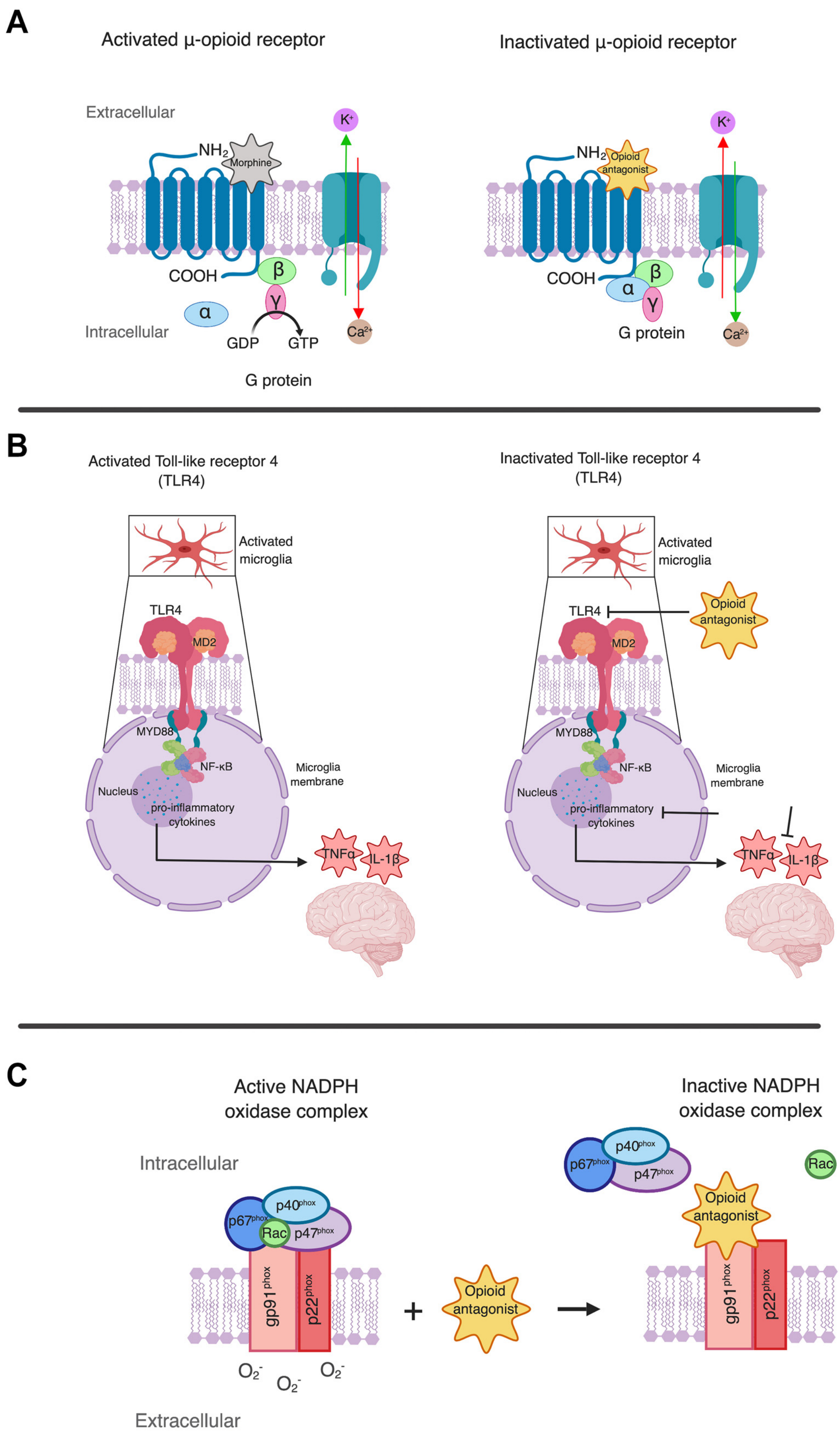

Fig. 3. Suggested neuroprotective mechanisms for opioid antagonists. While the neuroprotective mechanisms of opioid antagonists are not clearly understood, the following mechanisms are being considered. **A.** μ-opioid receptors are 7 transmembrane spanning that activate G proteins composed of α, β and γ subunits which convert GDP to GTP. When activated, μ-opioid receptors exhibit inhibition of Ca^{2+} influx and activation of K^+ channels. Opioid antagonists block μ-opioid receptor activation by competitive binding. **B.** TLR4 signaling pathway is activated in microgliosis. As a result, neurotoxic mediators such as TNFα and IL-1β are released. Opioid antagonists are suggested to block TLR4 signaling, leading to inhibition of pro-inflammatory cytokine production of TNFα and IL-1β. **C.** NADPH (dihydronicotinamide adenine dinucleotide phosphate) oxidase is an enzyme complex involved in the induction of oxidative stress that consists a membrane bound $gp91^{phox}$ subunit and $p22^{phox}$ as well as three cytosolic proteins ($p40^{phox}$, $p47^{phox}$, and $p67^{phox}$). During an ischemic stroke, the NADPH complex is activated as and the cytosolic components are translocated to plasma membrane to interact with the membrane bound $gp91^{phox}$ subunit and $p22^{phox}$ to assemble an active NADPH oxidase enzyme complex stimulating increased superoxide O_2^- generation. Opioid antagonists inhibit enzymatic activity of NADPH oxidase by binding to the $gp91^{phox}$ subunit and induce a conformational change of the NADPH protein complex affecting the binding affinity of the cytosolic subunits $p40^{phox}$, $p47^{phox}$, $p67^{phox}$. As a result, oxidative stress that compromises BBB integrity is reduced.

studied for improved stroke recovery and neuroprotective effects (Fig. 2, Table 2). As the κ receptor has been shown to be dysfunctional following a CNS injury, studies have employed nalmefene hydrochloride for acute ischemic stroke treatment due to its κ opioid receptor antagonist properties (Clark et al., 1996). To date, the effects of nalmefene, commercially sold as Cervene, is not fully understood in human ischemic stroke patients. In a pilot study, the efficacy of Cervene was compared to placebo in a randomized double-blind clinical trial.

Specifically, 34 ischemic stroke patients received 0.05 mg/kg of Cervene intravenously for 15 min and then were transferred to a dosage of 0.01 mg/kg for 24 h. A control group of 10 ischemic stroke patients that received placebo was maintained as well. Cervene efficacy was assessed by comparing the patient's National Institutes of Health Stroke Scale Score (NIHSS) at baseline to scores 7 days after treatment. Glasgow Coma Scale (GCS), which is a measure of recovery from brain injuries, were obtained 3 month after as a secondary efficacy measure (McMillan et al., 2016). Results indicated that while statistically significant efficacy of Cervene cannot be deduced from this small scale study, this opioid antagonist is safe and tolerable, and may be a beneficial stroke treatment for neurological recovery and improved functional recovery (Clark et al., 1996) (Table 2). Another study observed the neuroprotective effects of Nalmefene in patients with cerebral infarctions as large cerebral infarctions often lead to hypoxia, ischemia, and necrosis (Li and Song, 2017). Specifically, 236 patients with middle cerebral artery trunk infarction were randomly divided into two groups: a control group receiving conventional treatment and an experimental group receiving 0.2 mg of intravenous Nalmefene hydrochloride injections twice per day for 10 days (Li and Song, 2017). Patient treated with nalmefene had significantly low NIHSS scores in comparison to control group patients with large cerebral infarction. Similarly, there was a statistically significant difference between GCS scores of patients in the nalmefene treatment group in comparison to those in the control group (Li and Song, 2017). However, the long-term therapeutic efficacy of Nalmefene was not studied and cannot be concluded from this study. Indeed, as only few clinical studies with Nalmefene have been conducted, the therapeutic efficacy and ability to restore neurologic function remain largely unknown.

4. Future directions

Current stroke treatment is limited to only one FDA-approved drug, tPA. Efficient tPA use is limited to 3% of patients and has no apparent neuroprotective or neurological recovery effects. There is a need for novel drugs and drug delivery to promote stroke recovery through protection of the BBB. While further human and animal studies need to be conducted to evaluate therapeutic efficacy and more clearly understand mechanism, the use of opioid antagonists as a potential therapeutic agent for ischemic stroke suggests a novel repurposing of FDA-approved opioid antagonists that should be further explored.

Future work should study the mechanism by which these opioid antagonists are inducing neuroprotective and neurorestoration effects. By better understanding these drugs mechanistically, drugs with similar mechanism of actions may also be explored for their protective effects. Simultaneously, to date, there are no *in vitro* studies observing the effects of naloxone, naltrexone, and nalmefene on an *in vitro* model of ischemic stroke. Therefore, in addition to further *in vivo* studies evaluating the mechanism and further evaluating the therapeutic efficacy of these opioid antagonists as agents for stroke, future studies should also include in vitro studies as additional studies that may shed light on the mechanism by which these drugs are inducing their neuroprotective or neurorestorative effects. Similarly, while naloxone, naltrexone, and nalmefene are the only FDA-approved centrally activated opioid antagonists, it may also be beneficial to explore peripherally activated opioid antagonists for any neuroprotective effects. Nevertheless, long-term studies should be conducted to not only ensure the efficacy of these drugs in their neuroprotective properties, but to also ensure that these drugs have no negative side-effects, including toxicity, with long term use. Various treatments regimes and dosage should also be evaluated to determine the most effective treatment plans.

As opioid antagonists should be further studied for their potential as stroke therapeutics, it is also important to draw attention to the need for enhancing the deliveries of these opioid antagonists through use of novel drug delivery strategies and state of the art drug designs. Many promising neuroprotective agents have failed in clinical trials due to safety or efficacy (Panagiotou and Saha, 2015). Drugs are most commonly administered via oral delivery or as an injection. As a result, the drug may have off target effects by affecting healthy cells and organs as well (Singh et al., 2011). Simultaneously, drug efficacy is lost as the majority of the drug may be metabolized by other organs such as the liver, with a small dose reaching the organ of interest (Gavhane and Yadav, 2012). Consequently, a higher dose of the drug is needed to make up for the low bioavailability of injections or oral delivery. One major hurdle for targeting a drug to the brain is the highly restrictive BBB, especially for non-invasive transport of drug to the brain. Oral delivery of drugs poses many issues including low bioavailability, slow absorption, hepatic first-pass metabolism, and gastrointestinal (GI) side effects (Salatin et al., 2016). Many of the current drug delivery strategies utilized in the above studies to enhance drug permeability through the BBB are invasive including intraventricular or intracerebral infusion of the drug. These techniques are high risk and can have many dangerous complications for the patient (Wohlfart et al., 2012).

One way to overcome the need for very invasive drug delivery, such as intracerebral drug infusion is to enhance the design of stroke therapeutics, *i.e.* the prospective opioid antagonists, for more effective passage across the BBB. Nanotechnology is an innovative form of drug development that can be used to enhance the delivery of opioid antagonists for stroke therapeutics through optimization of various characteristics of the drug molecule shape and size to achieve a nanoparticle formulation of the opioid antagonists that is lipid soluble, has a low molecular weight, and is small in size, in turn enhancing the delivery of the drug across the BBB (Dong, 2018).

Nanoparticles are solid colloidal particles that can be controlled to be very small in size to freely cross the BBB while not disturbing BBB integrity (Panagiotou and Saha, 2015; Singh et al., 2011). The goal of developing a drug into nanoparticles is to ensure release of drug at a specific rate, dose, and site (Singh and Lillard, 2009). Nanotechnology based drug delivery offers localized, controlled, and sustained drug delivery, in turn increasing the therapeutic efficiency of the drug, reducing dosage and frequency of doses, as well as reducing off target effects to other organs and cells (Singh et al., 2011). Due to the reduced particle size and decreased diffusion distance, nanoparticles offer faster and more effective drug absorption. The small particle size provides increased contact area, allowing for increased drug adhesiveness to the cell surface, in turn, increasing drug bioavailability (Junyaprasert and Morakul, 2014). Nanoparticles preserve the innate therapeutic and non-toxic properties of original drugs while increasing bioavailability in comparison to traditional drug delivery forms. Therefore, dosage and frequency of dosage is decreased (Junyaprasert and Morakul, 2014). Simultaneously, the therapeutic effects of the original drug are preserved.

Composition of nanoparticle surface has been studied to be critical when targeting the brain. Nanoparticles fabricated with nonionic surfactants have been shown to exhibit increased uptake by the brain and more successful passage through the BBB. Other strategies such as use of viral vectors and exosomes have also been studied for brain drug delivery, however may not be effective strategies for ischemic stroke brain drug delivery (Dong, 2018). Viral vectors, for example, are beneficial for transfecting genes to patients that cannot normally cross the BBB. However, they have many limitations including patient safety and production costs as well as invasive administration routes such as injection into the cerebrospinal fluid (Dong, 2018). Exosomes are another drug delivery technique that involves the use of cell vesicles as a carrier for brain drugs delivery (Dong, 2018). Exosomes have often been studied for brain gene delivery, transporting proteins and nucleic acids across the BBB. As exosomes are non-immunogenic, they allow for enhanced circulation of the drug or protein of interest. However, exosomes also have many limitations including selection of the exosome carrier cell and vesicle loading (Dong, 2018). Further toxicity studies also need to be conducted with exosomes.

Nanotechnology has the potential to enhance potential stroke recovery therapeutics, such as opioid antagonists, and their respective passage of the treatment across the BBB to achieve a more direct delivery to the brain. As a result, a significantly invasive delivery (*i.e.* cerebral infusion) will not be necessary for successful drug administration (Saraiva et al., 2016; Chen and Gao, 2017). Concurrently, nanoparticles offer many advantages to traditional drug delivery systems, including increased drug solubility, bioavailability, and therapeutic efficacy. Nanoparticles may be a plausible future development in drug delivery methods of opioid antagonists, with the goal to ultimately improve patient outcomes.

Future human and animal studies should generate new knowledge to further understand the therapeutic efficacy and cellular and molecular mechanisms underlying the effectiveness of opioid antagonists for their potential in attenuating stroke severity, promoting recovery, and protecting the BBB against opioid-associated cerebrovascular complications. Progress in drug delivery methods to enhance these prospective stroke recovery treatments is suggested, with the ultimate goal of improving the lives of patients and their recovery from ischemic stroke.

Declaration of Competing Interest

The authors' state that they have no competing financial interests.

Acknowledgements

This work was supported by the National Institutes of Health (R01GM114321, R01GM127706, R01MH104656, R01MH110415, HL126559, DA039576, DA040537, and DA044579) and the National Science Foundation (CHE-1506740, CBET-1841419). SD thanks the Miller School of Medicine of the University of Miami for the Lucille P. Markey Chair in Biochemistry and Molecular Biology.

Appendix A. The Peer Review Overview

The Peer Review Overview associated with this article can be found in the online version, at doi:https://doi.org/10.1016/j.pneurobio.2019.101679.

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