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# Global Cerebral Ischemia: Synaptic and Cognitive Dysfunction

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## Abstract

Cardiopulmonary arrest is one of the leading causes of death and disability, primarily occurring in the aged population. Numerous global cerebral ischemia animal models induce neuronal damage similar to cardiac arrest. These global cerebral ischemia models range from vessel occlusion to total cessation of cardiac function, both of which have allowed for the investigation of this multifaceted disease and detection of numerous agents that are neuroprotective. Synapses endure a variety of alterations after global cerebral ischemia from the resulting excitotoxicity and have been a major target for neuroprotection; however, neuroprotective agents have proven unsuccessful in clinical trials, as neurological outcomes have not displayed significant improvements in patients. A majority of these neuroprotective agents have specific neuronal targets, where the success of future neuroprotective agents may depend on non-specific targets and numerous cognitive improvements. This review focuses on the different models of global cerebral ischemia, neuronal synaptic alterations, synaptic neuroprotection and behavioral tests that can be used to determine deficits in cognitive function after global cerebral ischemia.

### Keywords

Aging; cardiac arrest; global cerebral ischemia; hippocampus; neuroprotection; synapse

## INTRODUCTION

Cardiopulmonary arrest is a major cause of death and disability in the United States, as approximately 250,000 – 300,000 people experience sudden cardiac arrest each year [1, 2]. Despite improved emergency treatments (i.e. therapeutic hypothermia [3]) and resuscitation techniques, the survival rates after out-of-hospital cardiac arrest (CA) remains around 8%

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**CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflicts of interest.

and have not changed for the past 3 decades [4-7]. Epidemiological studies suggest that the risk of CA is higher among the aged population (51.1% over 65 years of age) as compared to the young (18.7% younger than 34 years old), with a mean age around 64 years old [4, 8-10]. CA can result from numerous conditions including: ventricular fibrillation, hypertrophic cardiomyopathy, arrhythmias (e.g., long QT syndrome or Wolff-Parkinson-White), coronary abnormalities, aortic rupture, arrhythmogenic right-ventricular cardiomyopathy and myocardial infarctions (artery blockage) [11, 12]. Ventricular fibrillation is the most common cause of CA in the aged population, whereas CA in the young (under 30 years) is commonly experienced during physical activity due to an inherited heart condition [11, 13].

The main consequence of CA is that it limits cerebral blood flow resulting in low oxygen and glucose levels. This decreases mitochondrial ATP production [14] and results in the accumulation of metabolic by-products [15]. The brain is one of the first organs to sustain damage from global ischemia, due to the high energy consumption and electrical activity. This ischemia-related decrease in ATP production results in numerous consequences, including the loss of ion gradients [16], release of excitatory neurotransmitters (i.e. glutamate), increase in intracellular calcium ($Ca^{2+}$) levels, and neuronal excitotoxicity [17].

This review will provide an overview of global cerebral ischemia: 1) the various models of global cerebral ischemia; 2) the ensuing neuronal synaptic alterations; 3) synaptic neuroprotection; and 4) the behavioral tests that can be used to determine deficits in cognitive function, which is a consequence of CA.

## AGING AND CA

The incidence of sudden CA is less than 70 per 100,000 individuals aged 32–36 years and steadily increases to over 700 per 100,000 individuals over the age of 82 [4, 10], where this increased rate of CA is similar among races and sex [8, 9]. Epidemiological studies have underscored the importance to study aged animals to better correlate research findings to the human population. Below we present a correlation between human lifespan and that of rats, as they are the most popular and suitable model for *in vivo* global cerebral ischemia studies.

A few studies have examined growth curves and survival characteristics of various rat strains, where the lifespan of laboratory rat varies from strain to strain and varied between strains sex [18, 19]. For example, Brown Norway/RijNia rats survive an average of 1200 days, while the lifespan of Fisher 344-NIH rats are reduced by 20% [18], and Wistar rats have relatively short lifespan of 600–700 days [19]. Turturro *et al.* also noticed that survival curves for gender were non- significant for Brown Norway/RijNia rats; however, female rats were right-shifted in Fisher 344-NIH rats and left-shifted in a hybrid strain derived by crossing Brown Norway/RijNia and Fisher 344 rats as compared to male rats [18]. The dilemma in the aging field has been how to compare the age of rats to humans. Quinn indicates that a simple conversion of average human lifespan to rat lifespan may not be appropriate for all ages, because the rate of development in humans and rats contrast during different stages of life [20]. For example, the period of birth to weaning is relatively longer in rats compared to humans, while the period of birth to musculoskeletal maturity is

relatively shorter in rats compared to humans [20]. Similarly, the post-senescence period in rats is relatively short compare to that in humans [20]. Investigators studying the process of aging in rodents consider 50% survival as the correlative age for old humans [21, 22], and as per this criterion, a 24 month-old Fisher 344 rat is considered as an aged rat.

The Stroke Therapy Academic and Industry Roundtable (STAIR) preclinical recommendations have emphasized the importance of testing neuroprotective therapies in aged animal models [23]. However, most studies examining the effects of CA on the brain use 3-4 month-old rats, and only a few studies have used aged rats [24-26]. Poor post-CA survival is one of the major factors discouraging investigators from performing studies in aged rats. Xu *et al.* observed that 4 day post-CA survival in 24 month-old rats is less than 40% compared to approximately 70% survival in a 6 month-old rats [26]. The presence of age-related diseases prior to the induction of CA and relatively poor recovery may account for poor survival in aged rats. Furthermore, another drawback to aged animals is that a 24 month-old Fisher 344 rat is about 5 times more costly than a 3 month-old one [27]. Another issue with using aged animals is that normal aging can result in cognitive deficits (for review see [28]), which must be taken into account when designing experiments for age appropriate models of global cerebral ischemia. For example, working memory deficits (see cognitive dysfunction section) have been reported in aged animals on the delayed alternation task [29]. These deficits in aged animals emphasize the importance of using aged matched controls and the potential for inherent variability when examining cognitive dysfunction after global cerebral ischemia. Overall, the epidemiological studies from humans suggests increased incidence of CA in the aged population, where characterizing/developing an appropriate animal model of CA with decent long-term survival remains a major challenge in the field.

## MODELS OF CA-INDUCED GLOBAL CEREBRAL ISCHEMIA

Numerous animal models of global cerebral ischemia have been developed over the years to induce ischemic neuronal damage [30]. These global cerebral ischemia models have an important role in furthering the understanding of how this disease affects the brain, and in developing therapeutics to minimize/hinder neuronal damage. There are two types of animal models that are commonly used to study the effects of CA-induced global cerebral ischemia. The first method of CA is through the interruption of cerebral blood flow (similar to what would occur during CA), whereas the second method is induced by inhibiting systemic circulation. These models mimic the ischemic conditions of cardiac arrest in young and aged humans irrespective of the conditions/diseases that induce CA. Below is a summary of these two animal model types, with their advantages and limitations.

The two- and four-vessel occlusion models are the most common rodent models of global cerebral ischemia [30, 31]. These vessel occlusion models induce a “square-wave” type (rapid loss and re-installment of blood flow) of ischemic insult in the forebrain without directly affecting other organ systems. The two-vessel model is induced by occluding both common carotid arteries in combination with controlled arterial hemorrhage-induced hypotension (50 mmHg) [30, 31]. This technique is predominantly performed in rats to reduce cerebral blood flow to less than 15% in brain regions such as cerebral cortex, caudoputamen, hippocampus, and cingulate cortex [30]. However, the use of anesthetized

and artificially ventilated animals prevents investigators from assessing neurological score/behavior during cerebral ischemia or early reperfusion [30, 31]. Additionally, the two-vessel occlusion model has been mimicked in mice by withdrawing blood from the jugular vein to induce hypotension [32]. On the other hand, the four-vessel occlusion model can be induced in awake, freely moving rats to assess neurological scores/behavior. Four vessel occlusion is performed in two phases, where the two vertebral arteries are permanently occluded by electrocoagulation and atraumatic arterial loops are placed around both common carotid arteries while the animal is under anesthesia [30, 31]. The animal is allowed to recover for at least one day and then, while awake, both common carotid arteries are occluded by tightening atraumatic arterial loops. This procedure reduces cerebral blood flow to less than 7% in striatum, neocortex and hippocampus [30]; however, poor survival and permanent ligation of vertebral arteries are major limitations of the four-vessel occlusion model.

Several models are available for whole-body ischemia, which either inhibit cardiac contractions or reduce systemic circulation. One of the most widely used models of CA is the rapid intra-arterial injection of cold potassium chloride, with the cessation of ventilation, to induce asystole [33-36]. In this model a “square-wave” insult (rapid cessation and initiation of blood flow) is produced in anesthetized animals and allows for the absence of mechanical or electrical damage to the heart. After a specified duration of CA, cardiopulmonary resuscitation is achieved through chest compressions and intravenous delivery of epinephrine. One potential concern for this method is the unknown effects that high concentrations of potassium chloride have on organs, including the brain. Another limitation is the requirement to maintain cerebral temperature in mice around 40°C or increasing the duration of CA to 12 min for histopathological changes in one of the most susceptible regions of the brain (i.e., hippocampal neurons) [33, 35].

Another method of CA is to induce ventricular fibrillation by delivering alternating current to either the right ventricular endocardium, through transoesophageal cardiac pacing, or transthoracal electrical fibrillation [37-40]. Animals are resuscitated by delivering a countershock of direct current along with chest compressions and intravenous delivery of epinephrine. These models are difficult and require considerable technical skills; however, they are beneficial for studying short-term recovery, as survival remains an issue for long durations.

An additional method of CA is through asphyxia, which is a relatively simple model and can be achieved after some practice [41, 42]. Apnea is commonly used to induce asphyxial CA by disconnecting the ventilator from the endotracheal tube of a pharmacologically paralyzed mechanically ventilated animal. Again after a specified duration of CA, cardiopulmonary resuscitation is achieved through chest compressions and intravenous delivery of epinephrine. The disadvantage of this method is that the time to “no blood flow” requires several minutes and does not produce a “square-wave” type insult [41, 42]. Finally, cessation of the systemic circulation can be performed by “continuous chest compression” or “compression of the heart vascular bundle against the sternum by use of a microsurgical hook”; however, these are less popular methods [43-45]. Both methods mechanically prevent the heart from pumping blood throughout the body to mimic global ischemia.

In general, the differences in anatomy of the cerebrovascular systems among different rodents can result in differential brain damage pattern following global cerebral ischemia using two vessel occlusion model [30]; however, these anatomical differences should not be problematic using whole-body ischemia models. While all of these global cerebral ischemia models are commonly used on small animals, the STAIR criteria has encouraged follow-up studies in larger animals with a gyrencephalic brain before any therapeutic agents are advanced into clinical trials [23]. Induction of CA in large animals such as dogs and pigs is routinely performed using ventricular fibrillation, rapid intra-arterial injection of potassium chloride, or asphyxia [46-53]. Overall, the proper model of global cerebral ischemia should be selected based upon on the hypothesis being tested.

## SYNAPTIC MODIFICATIONS AFTER GLOBAL CEREBRAL ISCHEMIA

### A) Ion Channel Conductance Changes

Global cerebral ischemia is a multifaceted disorder that affects the entire brain; however, the majority of research has focused on the hippocampus, as this important region is one of the areas affected Fig. (1) [54, 55]. Each CA1 neuron of the hippocampus has an estimated 30,000 excitatory and 1,700 inhibitory synaptic inputs Fig. (2) [56]. This enormous amount of excitatory connections is one factor that increases the sensitivity of the CA1 neurons towards ischemia [57]. In general, CA1 neurons respond to ischemia in different phases, where initially the onset of oxygen/glucose deprivation (OGD) is associated with a small neuronal depolarization and increase in excitability [58-60]. This phase is superseded by a reversible hyperpolarization phase from an increase in $K^+$ conductance [58-61]. This hyperpolarization phase consists of different types of $K^+$ channels, including the $Ca^{2+}$-activated $K^+$ channels and ATP-sensitive $K^+$ ($K^+_{ATP}$) channels [60]. This hyperpolarization phase is then proceeded by a large depolarization that spreads to the surrounding neurons (hypoxic or spreading depression), stimulating a massive glutamate release [59, 62].

### B) Synaptic Changes After Global Cerebral Ischemia

After ischemia, neurons can undergo apoptosis/necrosis or survive by maintaining cellular/ionic homeostasis [63, 64]. For neurons that undergo apoptosis/necrosis, $H_2O$ and $Ca^{2+}$ ions accumulate in the cytosol during the large depolarization phase, inducing neuronal swelling [65-69], mitochondrial swelling [65], and free radical mitochondrial damage [70]. Unlike other sub-regions of the hippocampus, CA1 neurons undergo delayed neuronal death, which can occur between 2-7 days after ischemia [55, 69, 71].

Non-apoptotic/necrotic neurons after ischemia undergo numerous modifications, including synaptic remodeling. Originally, pre-synaptic terminals were considered to be unaffected by ischemia [72, 73]; however, ischemia induces pre- and post-synaptic terminal alterations [74-77]. After ischemia, synaptic remodeling occurs immediately through an increased growth of filopodia, synapse formation, projection of spines and altered protein expression [68, 69, 74]. For example, synapsin-I (protein that dissociates synaptic vesicles from actin filaments) and phosphosynapsin immunoreactivitiy were increased after ischemia in CA1 pyramidal neurons [74, 78]; however, results from animals treated with middle cerebral artery occlusion have suggested that synapsin-I phosphorylation is decreased after ischemia

(phosphorylation dissociates vesicles from actin), producing a decrease in neurotransmitter release [75]. 24 hours after global cerebral ischemia *in vivo*, Yokota *et al.* used subtractive cDNA cloning to identify increases in the mRNA levels of synaptotagmin IV (synaptic vesicle integral membrane protein) [76]. Yet, there is a pre-synaptic reduction of the synaptic vesicle associated proteins (synaptosomal associated protein 25 and synaptophysin) two days following ischemia, which would reduce neurotransmission [79]. In the subsequent days following ischemia, numerous spines swell, become concave, have post-synaptic density thickening, pre-synaptic depletion of synaptic vesicle pools, decreased extracellular space and decreased synaptic spine density [65, 69]. There are also decreases in synapses, spines, total percent volume of axon terminals [77] and number of mitochondria per synapse in the CA1 region of the hippocampus [80].

Synaptic electrophysiological changes also occur after ischemia, which include an increased N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepro pionic acid (AMPA) receptor conductance [81-85]. This increased conductance has been described as a form of long-term potentiation (LTP) from the glutamate released during ischemia (iLTP) [86, 87]. The molecular mechanisms involved in iLTP are similar to those of traditional LTP, including the activation of NMDA receptors, $Ca^{2+}$ influx, calmodulin kinase 2 activation, and cAMP response element binding protein activation [82, 88] (reviewed in [86]). iLTP is also dependent on the activation of protein kinase C [89] and nitric oxide signaling [90]. One common neurological problem after global cerebral ischemia is seizures [54, 91-94]. Seizures have been attributed to an increase in hyperexcitability of CA3 neurons [95, 96], most likely from the resting membrane potential of CA3 neurons being permanently depolarized [95, 97]. This depolarized state could originate from a loss of inhibitory neurons in the dentate gyrus or CA3 region and an increase in excitatory glutamate stimulation [98-100].

Overall, global cerebral ischemia results in a massive glutamate release, stimulating numerous neurons. These neurons process this glutamate signal as an increase in excitatory output (LTP) and respond through an increased growth of spines, synaptic protein levels, and conductance of receptors [68, 69]. However, this massive glutamate release can also induce ischemic damage and result in delayed neuronal cell death, reducing the total number of CA1 hippocampal neurons and synapses.

## SYNAPTIC NEUROPROTECTION

### A) Synaptic Targets for Neuroprotection Post-Global Cerebral Ischemia

Rescuing neurons from delayed neuronal death has been a widely researched and therapeutic interest for neuroprotection. Neuroprotectants are agents that antagonize, halt, or impede the molecular and biochemical events that induce ischemic damage. Numerous agents afford neuroprotection in animal models by modulating cerebral blood flow, including thrombolytics [101, 102], anti-thrombotytics [103, 104], anti-platelets [105, 106], and fibrinogen-depleting agents [107]. Hypothermia, the only widely clinically used method of neuroprotection after ischemia [108, 109]; reviewed in [110], improves cortical blood flow [111, 112], decreases neuronal activity and oxygen consumption [113], reduces free radical production [114], and reduces inflammatory signaling for neuroprotection [115]. These

agents afford neuroprotection through increased blood flow, oxygen/glucose levels, and clearance of reactive oxygen species; however, the evidence of synaptic alterations of these agents are still preliminary [116, 117].

One heavily researched area of synaptic neuroprotection after cerebral ischemia is the glutamate activation of NMDA and AMPA receptors, and the resulting excitotoxicity from the increased cytosolic $Ca^{2+}$ levels Fig. (3). While numerous agents exhibit successful NMDA-dependent neuroprotection in experimental animal models, adverse side-effects in humans have limited their clinical use [118-123]. Another problem for these agents has been the limited testing on young/juvenile animals, where neuroprotectants have been less effective in aged animals [124]. The failure of numerous neuroprotectants in clinical trials has been comprehensively reviewed previously [120]. A few of the most important features in these trials were the usage of doses in humans that were significantly lower than those that provided neuroprotection in animal models, the advancement to clinical trials without solid pre-clinical testing, and the failure to follow the STAIR criteria [125]. While clinical trials are a necessity before therapeutics can be used clinically, neuroprotectants should be comprehensively tested in animal models (*in vitro* and *in vivo*), display neuroprotection in aged animal models, have correlative behavior benefits (see behavior testing section below), display tolerable therapeutic dosages in humans, and identify a specified time window for treatment before advancement to clinical trials.

Another commonly investigated therapeutic method of neuroprotection is through increased neuronal inhibition after ischemia Fig. (3). γ-aminobutyric acid (GABA) receptor agonists have been used in animal models either by directly activating $GABA_A$ [126-130] or $GABA_B$ receptors [129, 130], and by indirect modulation of $GABA_A$ receptors [131]. While increased GABA receptor activation immediately after ischemia has displayed neuroprotection in animal models, reduced GABA-mediated tonic inhibition 4 days after focal ischemia has been suggested to promote recovery [132]. This delayed GABA receptor inhibition is most likely neuroprotective as inhibitory synaptic transmission is increased after ischemia [133]. Together, these data suggest that modulating GABA-related currents may require initial activation and then antagonism days after the ischemic event may be required for neuroprotection.

While targeting an individual receptor can be beneficial, the usage of nonspecific therapeutic agents can afford neuroprotection through the modulation of multiple targets. For example, the administration of resveratrol during OGD decreased cellular depolarization and number of excitatory postsynaptic currents, whereas after OGD, action potential frequency was decreased [134]. Part of this neuroprotection was attributed to (but not limited to) the modulation of $Ca^{2+}$- activated $K^+$ channels, thereby reducing the amount of action potentials generated by glutamate stimulation. Therefore, targeting synaptic receptors (either during or immediately following ischemia) may require the administration of nonselective or multi-target agents for neuroprotection, as a single agent may only be neuroprotective to a limited neuron population.

### B) Synaptic Targets for Neuroprotection Pre-Global Cerebral Ischemia

While the administration of therapeutic agents after global cerebral ischemia is a major target for neuroprotection, there is a large population who may be at risk for cerebral ischemia and would benefit from "preconditioning" (i.e., recurrent ischemic events [8, 135] or high-risk individuals [136, 137]). Preconditioning is the activation of protective cellular mechanisms, whereby the tissue is protected against future ischemic events (hours to days). The first mechanism of preconditioning described was ischemic preconditioning (IPC), where a sublethal ischemic insult protects neurons against a subsequent lethal insult [138, 139]. Numerous drugs/compounds have the ability to precondition neurons by activating pathways similar to IPC (Table 1).

Numerous studies have indicated the involvement of NMDA receptor activation or modulation as a prerequisite for preconditioning [140-146]. Preconditioning-induced activation of synaptic NMDA receptors (not AMPA receptors) [141] activates pro-survival extracellular signal-regulated kinase [145], increases brain-derived neurotrophic factor release [147, 148], decreases heat shock protein 70 [149], and decreases glucose-regulated protein 94 for neuroprotection [149]. However, not all researchers have found this NMDA receptor dependence [150] or have suggested that NMDA receptors activation is only one pathway of IPC-induction [151]. The differences found between these experimental outcomes may rely on the type of preconditioning used, where IPC may induce numerous modifications for preconditioning and may not be exclusively dependent on NMDA receptor activation.

Beyond NMDA receptor activation, numerous synaptic targets (direct or indirect) can induce neuroprotection Fig. (3). For example, treatment with plasmalemma $K^+_{ATP}$ channel agonist (i.e., (−)cromakalim) 3 days prior to *in vivo* global cerebral ischemia induced neuroprotection in the CA1 and CA3 region of the hippocampus [152]. Another study suggested that preconditioning with 3-nitropropionic acid (mitochondrial respiration chain inhibitor) modulates synaptic $K^+_{ATP}$ channels to delay the onset of hypoxic depolarization, decreasing the excitotoxic glutamate release for *in vitro* neuroprotection [153]. Additionally, adenosine A1 receptor agonists (e.g., $N^6$-cyclopentyladenosine) induce immediate neuroprotection through $K^+_{ATP}$ channel opening [154-156]; however, *in vivo* administration of adenosine A1 receptor agonist 3 days prior to global cerebral ischemia induced neuroprotection through enhanced expression of heat shock protein 70 [152], suggesting ubiquitous preconditioning mechanisms of the adenosine A1 receptors. Finally, $Ca^{2+}$-activated $K^+$ channel agonist 1-ethyl-benzimidazolinone administered 30 min before global cerebral ischemia induced neuroprotection by maintaining of $Ca^{2+}$-activated $K^+$ channel expression and activity [157].

Another preconditioning method is the activation of epsilon PKC (εPKC), a novel PKC activated by IPC [146, 158]. εPKC preconditioning increases the average amplitude of GABA-related inhibitory post-synaptic events [159], maintains ion homeostasis during ischemia by inhibiting $Na^+/K^+$-ATPase and voltage gated $Na^+$ channels [160], improves cerebral blood flow [161], and phosphorylates the mitochondrial $K^+_{ATP}$ channel [162]. The plethora of responses that are produced from εPKC activation indicates the necessity for a preconditioning agent to have numerous targets for neuroprotection or IPC mimetic. This

would allow for numerous systems to be modulated/activated, beyond a specific cellular target, to induce neuroprotection.

## GLOBAL CEREBRAL ISCHEMIA AND COGNITIVE DYSFUNCTION

CA and the resulting prolonged transient global cerebral ischemia result in a multitude of cognitive dysfunctions in humans, including deficits in memory [163-166], executive function [163, 167], motor function [163, 166], and anxiety [163, 166, 168, 169]. In humans, the behavioral outcomes after CA vary depending on the type and length of ischemic injury varying within the individual; nevertheless, some aspects of cognitive dysfunction post-global cerebral ischemia have been characterized. The most susceptible regions of the brain to global cerebral ischemia are the CA1 region of the hippocampus [55, 164, 165], cortex [55, 165], prefrontal cortex (PFC) [165], thalamus [165], cerebellum [165], and putamen [55]. Unfortunately, a majority of these studies in humans have a small sample size or are retrospective studies, limiting the impact of the findings. Another issue is the wide spread nature of global cerebral ischemia, which limits a researcher's ability to dissect apart individual brain regions and determine which damaged region is responsible for specific cognition deficits. Therefore, in order to separate apart the extent of ischemic damage and which brain region is responsible for each cognitive deficit, studies using animals have been a necessity.

### A) Spatial Memory

One of the most well-defined systems of memory in both humans [163] and animals [170, 171] is spatial memory. Spatial memory is the ability to learn a surrounding environment and orientation, where this ability is impaired with lesions of the hippocampus [172] or entorhinal-perirhinal cortex (hippocampus projection to the neocortex) [173]. In animals, a common method for testing spatial memory is the Morris water maze [174], which involves placing the animal (commonly rodents) in a pool of opaque water with a hidden underwater platform. The animal navigates the pool using spatial cues located outside the pool to find the correct quadrant where the platform is located. The animal's ability to learn the location of the platform is tested over a period of days, allowing for the animal to recall the platform location on subsequent trials. Deficits in spatial learning are determined by the time the animal takes to locate the platform and the overall distance traveled. For example, Morris water maze performance after global cerebral ischemia is impaired with an inability to locate the platform compared to sham controls [175, 176]. This deficit has been attributed to a decreased dendritic spine density and cell death of CA1 neurons [176-178].

While the Morris water maze can be a useful technique to determine spatial memory deficits, this test requires animals to swim and could be an issue for animals that suffer from motor dysfunction after cerebral ischemia [179-181]. Therefore, the Morris water maze may be an unsuitable model for testing animals with impaired motor function. Due to this disadvantage, alternative spatial memory tasks that are less physically demanding are the Barnes circular platform maze [182] and contextual fear conditioning [183]. In the Barnes circular maze, the animal is tasked with finding a hole on the outside of a circular platform that contains a dark box using spatial cues (similar to the Morris water maze) and deficits in

spatial memory develop with the number of errors and overall distance traveled to locate the correct hole Fig. (4A) [182]. Contextual fear conditioning measures freezing behavior of animals when returned to an environment where an electric shock was delivered [184]. Overall, the Barnes circular maze and contextual fear conditioning spatial memory tasks may be more suitable in animal models with motor deficits, due to the reduced motor requirements to complete the tasks.

### B) Executive Function

Executive function is a set of neurological processes that control and regulate the ability to organize thoughts, perform tasks, and make decisions. One well defined type of memory required for executive function in humans [185] and animals [170] is working memory; the ability to remember (for a brief period of time) items or facts that are distinguishable from previously learned information [186]. Working memory is dependent on the PFC in humans [187] and rodents [188]. After global cerebral ischemia, damaged has been reported in the PFC in both humans [165] and rodents [189, 190]. Damage to the PFC [191, 192], hippocampus [193-196], entorhinal cortex [197], anterior thalamic nuclei [198], and septum [199] have been suggested to inhibit learned alternation. Learned alternation is the ability for a rodent to alternate from a normal task to obtain a reward, where this is commonly performed using the T or Y maze alternation task Fig. (4B) [200]. In the T or Y maze alternation task, the animal is placed at one end of the maze and trained to turn a specific direction (at the branch) to obtain a reward (normally food). On subsequent trials, the animal is only rewarded if it learns to travel down the opposite arm from where it originally received the award. This procedure is also referred to as a “win-shift strategy”, because the animal only correctly completes the maze if it shifts away from the previous location of the reward. Another test to examine working memory is the delayed matched to sample water maze, a modified version of the Morris water maze that moves the escape platform each day [186]. This constant movement forces the animal to learn the new location of the platform each day, while separating out previously learned locations. The delayed match to sample water maze thought to be dependent on PFC function and may be capable of separating out differences between spatial memory and working memory differences [186].

Working memory after global cerebral ischemia in humans has led to a mix of reports, as some investigators suggest a decrease in working memory performance [163, 167] and others were unsuccessful at identifying working memory deficits [201]. Interestingly, minimal work has characterized executive function deficits after global cerebral ischemia in rodents, in comparison to other types of cognitive dysfunction (e.g. spatial memory or motor function). One study has indicated a deficit in T-maze alternation task months after global cerebral ischemia; however, these results are difficult to interpret as working memory deficits and motivation of these animals were also affected [202]. Although T-maze performance is altered with PFC damage [191], this task maybe dependent on additional brain structures that are affected by global cerebral ischemia (i.e. hippocampus [193]), further confounding the relationship between PFC damage and working memory performance. Currently, studies on animals after global cerebral ischemia in the delayed match to sample water maze have not been implemented, and may have unforeseen drawbacks. The delayed match to sample water maze has similar drawbacks to the Morris

water maze, as performance may be compromised from motor impairments. However, the Barnes maze can be adapted to measure working memory [203], but, evidence linking performance on this task to PFC damage has not been estabolished. Overall, the assessment of working memory and other aspects of executive memory after global cerebral ischemia remains understudied and new techniques that accurately assess working memory in animals need to be developed.

### C) Motor Function

Global cerebral ischemia in humans can result in damage to the cerebellum [165, 204, 205], resulting in motor function impairments. Normally, gross motor function in humans can be observed using simple exams, such as the finger tap (participant taps their finger as fast as possible) [165] or grooved pegboard (participant places shaped pegs into a pegboard) [163]. After global cerebral ischemia, motor deficits have been reported on the finger tap [163, 166], and in severe cases, patients develop problems with gait [206]. In animals, motor function is commonly assessed using a few different techniques examining balance, coordination, and activity [207-210]. Locomotor function is frequently examined in rats using the beam walking task [208]. This test consists of an animal walking a narrow beam to escape an aversive stimulus, scoring the number of foot faults and amount of time it takes to cross the beam Fig. (4C). Global cerebral ischemia increases the amount of time required to complete this task, suggesting a deficit in motor function [207, 208]. The open field test is used to test overall activity in rodents [210], where an animal is placed into a square or circular arena (for anywhere between 5 and 30 minutes) and allowed to move freely. Generally, the amount of time the animal spends moving is an estimation of activity.

Results from different groups assessing locomotor activity using the open field test after global cerebral ischemia suggest that activity can be either increased or decreased, depending on the time after injury and age of the animal [202, 211, 212]. These results underscore the importance of when the test is conducted and the age of the animals being tested after global cerebral ischemia. A drawback of the open field test is that there is little assessment on gait and functional use of the animal’s limbs, which could be affected after global cerebral ischemia [179-181]. Also, while the beam walk test directly measures walking and balance, the test does not focus on more subtle changes that occur in limb coordination and range of motion in joints. Therefore, the beam walk test may not be as sensitive as other assessments of the locomotor function such as the Basso, Beattie, and Bresnahan locomotor score. This test is frequently used after spinal cord injury to examine more subtle changes limb coordination and range of motion in joints [213]. Another test that has shown to be more sensitive method to assess locomotor function is the tape removal test, where animals are timed in the removal of tape from both of their wrists [214, 215]. The tape removal test has been used after global cerebral ischemia previously, and may be a useful alternative to detect slight variations in sensory motor dysfunction [215]. Overall, conducting all the necessary cognitive tests to discern locomotor function after global cerebral ischemia can be a time consuming and difficult task to perform in potentially frail animals that have a short life expectancy.

### D) Depression and Anxiety

Depression after global cerebral ischemia is common among humans [163, 166], and is traditionally evaluated using a questionnaire [168, 169]. While identifying the cause of depression poses an exceptional challenge in healthy individuals, different brain regions that are damaged during global cerebral ischemia (i.e. PFC and hippocampus) have been implicated in depression (for review on regions and proposed mechanisms see [216]). Interestingly, comorbidity between anxiety and depressive disorders is very high, as the two disorders are thought to be closely linked [217]. In rodents, the open field test can be used to measure anxiety [218], where the number of times the animal enters the middle and the amount of time the animal spends in the middle of the box measure anxiety [218]. Another test measuring anxiety in rodents is the elevated plus arm maze [219], which uses an elevated platform in the shape of a plus symbol with two of the four arms containing high walls. The animal is placed into the middle of the maze and the amount of time the animal spends in the enclosed area versus the open area is calculated. Animals with high anxiety levels spend almost all of the time in the enclosed area [220], where rodents after global cerebral ischemia have demonstrated a decrease in anxiety [221, 222]. However, these animal results are confounding, as results from human studies suggest an increase in depression and anxiety [163, 166, 168].

Comparing anxiety and depression in rodents to humans is a very difficult task, because humans can verbally answer questions about their disposition and animals must be interpreted. Also, in order to clearly interpret anxiety and depression in rats, other behavior aspects (i.e. motivation and locomotor function) must be assessed to rule out the possibility of these confounding factors. Furthermore, the elevated plus maze has some inherent design flaws; for example, if the animal is unmotivated and remains in the center region for an extended period of time, the results can be difficult to interpret. This issue can be dealt with by using the zero maze [223], an updated version of the elevated plus maze. The zero maze solves this movement problem by using a circular design, where the starting position is not in the middle of the apparatus Fig. (4D). The zero maze has been repeatedly used to measure anxiety in rodents [223, 224] and may serve as a more evaluated alternative to the elevated plus maze. Another test of anxiety that measures anhedonia, a fundamental aspect of depression, is the preference for sugar water over regular water [225]. However, this model has not been used extensively in models of global cerebral ischemia. Overall, before depression and anxiety can be researched, problems in executive or locomotor function may need to be dissected to understand the significance of these results.

### E) Neuroprotection and Behavioral Assessment

While evaluating neuronal cell death after global cerebral ischemia is important for understanding how ischemia damages specific regions of the brain, the assessment of behavioral outcomes after neuroprotective therapies should be essential in demonstrating an improved neurological outcome. In humans, the most rigorously examined method of neuroprotection is hypothermia, however, these studies have shown minimal benefits in decreasing cognitive deficits in humans [167, 206, 226]. In animals, different neuroprotective treatments have exhibited varying results depending upon which cognitive aspects were tested. For example, IPC [227], pharmacological preconditioning [228, 229], or

ischemic postconditioning [230] have displayed improvements in spatial memory after global cerebral ischemia. Caloric restriction [171] and isoflurane preconditioning [231] before global cerebral ischemia displayed improvements in working memory. Repeated resveratrol preconditioning had improvements in attention and activity without benefits to working memory after global cerebral ischemia [232]. In general, neuroprotective therapies from global cerebral ischemia must use multiple behavioral tests to determine what aspects of cognitive dysfunction are benefited before clinical advancement.

## CONCLUSION

CA and the resulting global cerebral ischemia is a major cause of neurological dysfunction that normally occurs in the aged population. Numerous CA models have been developed to mimic this type of ischemia (vessel occlusion to cessation of the heart/blood flow) that has allowed researchers to investigate this multi-faceted disease; however, most of these studies have been limited to younger animals. Synapses endure a variety of alterations after global cerebral ischemia from the resulting excitatory glutamate stimulus and have been a major target for neuroprotection. However, numerous neuroprotective agents have been advanced into clinical trials targeting glutamate excitotoxicity or other synaptic components without significant improvements [120]. While most of these neuroprotective agents have had very specific neuronal targets, the future of neuroprotective therapies could depend on therapeutics that are pleiotropic in nature, such as the non-specific preconditioning agents (i.e. εPKC agonist) or post-ischemia (i.e. resveratrol), since they target multiple sites for neuroprotection. This would allow for numerous targets and pathways to be activated and limit the neuronal damage or protect CA1 hippocampal neurons from delayed neuronal cell death.

Furthermore, a shift in current models used to assess therapeutic neuroprotective treatments maybe necessary. Currently, increasing the number of healthy neurons has become the primary endpoint for studies investigating the efficacy of neuroprotectants; however, the future of these agents will also be dependent on their ability for clinically relevant behavior improvements. Therefore, future ischemia research should focus on characterizing cognitive deficits and how neuroprotectants reverse these behavioral deficits in addition to neuroprotection. The probability of global cerebral ischemia increases with age, suggesting the necessity for an aged-related clinically relevant model of ischemia [1, 2]. Although testing on aged animals may be difficult and costly, determining how aging effects global cerebral ischemia will be critical for designing treatments for aged populations.

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## ABBREVIATIONS

| | |
|---|---|
| **AMPA** | α-amino-3-hydroxy-5-methyl-4-isoxazole propionic Acid |
| **GABA** | γ-aminobutyric Acid |

| | |
|---|---|
| **ATP** | Sensitive $K^+$ - $K^+_{ATP}$ |
| **CA** | Cardiac Arrest |
| **$Ca^{2+}$** | Calcium |
| **CA1 or CA3** | Cornu Ammonis 1 or 3 |
| **DG** | Dentate Gyrus |
| **EC** | Entorhinal Cortex |
| **εPKC** | Epsilon PKC |
| **iLTP** | Ischemia Long-Term Potentiation |
| **IPC** | Ischemic Preconditioning |
| **LTP** | Long-Term Potentiation |
| **MF** | Mossy Fibers |
| **NMDA** | N-methyl-D-Aspartate |
| **OGD** | Oxygen/Glucose Deprivation |
| **PP** | Perforant Pathway |
| **PFC** | Prefrontal Cortex |
| **SC** | Schaffer Collateral Pathway |
| **STAIR** | Stroke Therapy Academic and Industry Roundtable |

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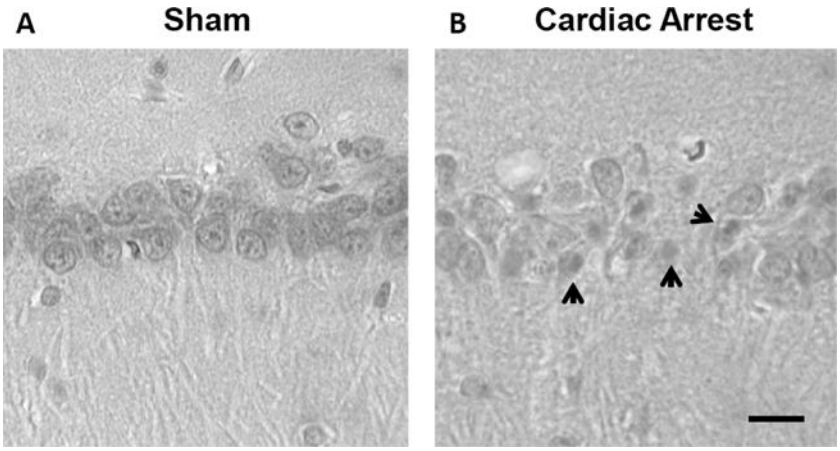


**Fig. (1). Histopathology changes after CA in CA1 region of the hippocampus**

Representative histological images of the hippocampal CA1 region after **A**) naïve control or **B**) 6 minutes of CA followed by 7 days of reperfusion. Ischemic neurons are indicated by arrowheads. All images were captured at 40X magnification and scale bar is 20 μm.

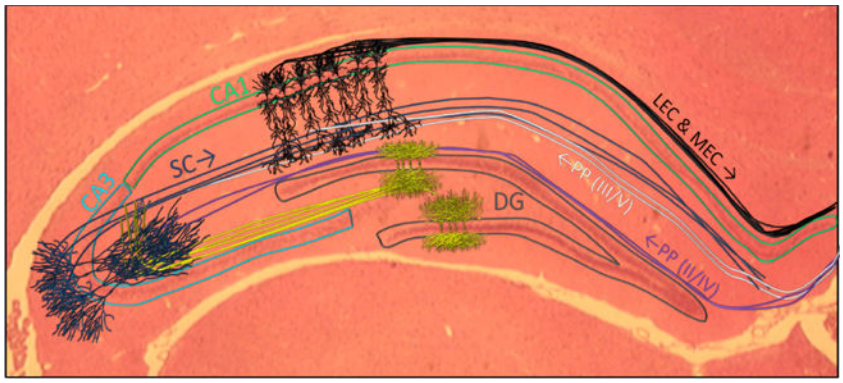


**Fig. (2). Circuitry of the Hippocampus**

Histological coronal slice image of the rat hippocampus with cartoon overlay depicting the hippocampal network. In general, the Entorhinal Cortex (EC) sends projections (via Perforant Pathway [PP]) to the Dentate Gyrus (DG) and CA3 pyramidal neurons (via PP from EC layers II/IV) or to CA1 neurons (via PP from EC layers III/V). DG sends projections to the CA3 neurons via Mossy Fibers (MF). CA3 neurons then relay the signal to CA1 pyramidal neurons via the Schaffer Collateral Pathway (SC). The CA1 neurons connect to the deep layers of the EC.

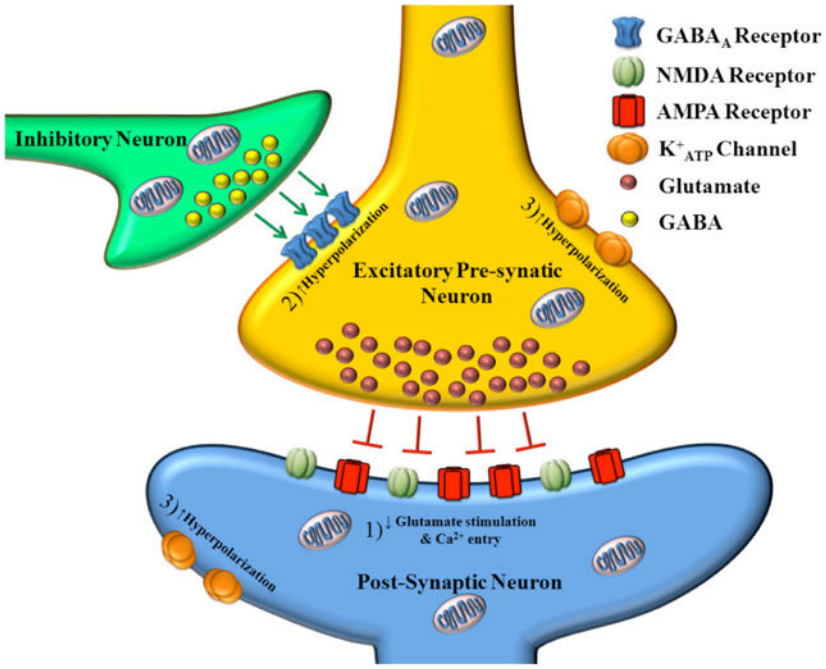


**Fig. (3). Synaptic targets for neuroprotection**

The primary excitatory neurotransmitter (i.e., glutamate) is released from presynaptic neurons upon an action potential, stimulating AMPA and NMDA receptors. Activation of these postsynaptic receptors initiates excitatory transmission and increases cytosolic $Ca^{2+}$ levels. Common targets for neuroprotection include: **1**) direct inhibition of NMDA and AMPA receptor activation to decrease excitatory transmission, **2**) increased inhibitory neuronal transmission (GABA receptor activity) to hyperpolarize and inhibit glutamate release. **3**) increased $K^+$ conductance for cellular hyperpolarization and decreased action potential propagation or post-synaptic depolarization.

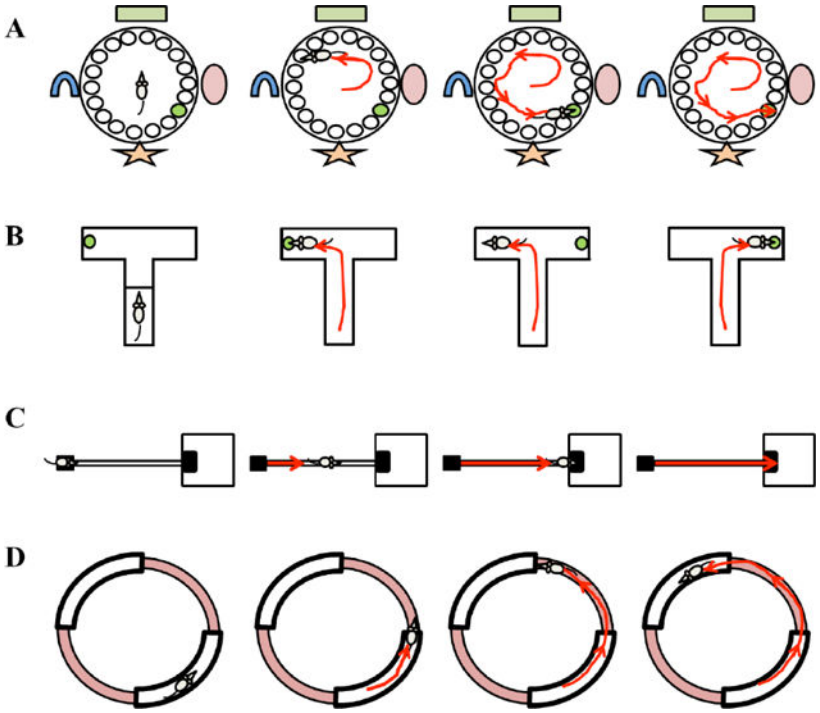


**Fig. (4). Cartoon representation of behavioral tests**

**A**) Barnes Maze: A rodent is placed in the center of the maze, uses spatial cues surrounding the maze (colored shapes) to search/locate the escape tunnel (green hole) and enter the escape tunnel to conclude the test. The number of visits to incorrect holes (empty holes) and distance traveled are used to determine spatial memory impairments. **B**) T/Y maze: A rodent is placed into the long arm of the maze and is prevented from leaving using a physical blockade, the blockade is removed and the rodent is trained to find a reward (green circle) consistently located in one arm. After training, the reward is shifted to the opposite arm and the rodent's ability to correctly locate the shifted reward is measured in subsequent trials to determine executive function impairments. **C**) Beam walk: A rodent is placed onto a small platform in front of a beam and progresses across the beam to enter a dark box. The time it takes the rodent to cross the beam/enter the box and the number of foot faults are measured to determine motor function impairments. **D**) Zero maze: A rodent is placed onto an area with enclosed walls (white). Over 5 minutes the number of times the rodent peers into the open area (red) and the amount of time the rodent spends in the open or closed area are measured to determine alterations in anxiety. [All images are sequential from left to right].

**Table 1**

Agents or Methods Used to Induce Preconditioning

| | |
|---|---|
| Adenosine Agonists | [152, 156] |
| Antibiotics | [233] (Reviewed in [234]) |
| ATP-sensitive $K^+$ ($K^+_{ATP}$) channel agonist | [152, 154, 155] |
| $Ca^{2+}$-activated $K^+$ channels | [157] |
| Diazoxide | [235] |
| GABA receptor antagonist | [236] |
| Epsilon PKC (PKC) agonist | [159] |
| Electroacupuncture | [237, 238] (Reviewed in [239]) |
| Electroconvulsive shock | [240] |
| Erythropoietin | [241-243] |
| Estrogen | [244, 245] |
| Ethanol | [246, 247] |
| Ginkgo biloba | [248] |
| Hypothermia or Hyperthermia | [249] |
| Hyperbaric oxygen therapy | [250, 251] |
| Iron chelators | [252, 253] |
| Ischemia | [155, 254] |
| Lipopolysaccharides | [255, 256] |
| $K^+$ channel agonists | [257, 258] |
| $K^+$ channel antagonist | [236] |
| Magnesium | [259] |
| Mitochondrial ATP $K^+$ channel modulators | [153, 235, 260] |
| 3-Nitropropionate | [261, 262] |
| NMDA | [146, 236] |
| Phosphocreatine | [263] |
| Phosphodiesterase III inhibitor | [228] |
| Remote preconditioning | [264-266] |
| Resveratrol | [267-269] |
| Statins | [270] |
| Succinic dehydrogenase inhibitor | [271] |
| Thrombin | [272, 273] |
| Toll-like receptor | [274-276] |
| Transient receptor potential melastatin 7 (hypothesized) | [184, 277] |
| Volatile anesthetics | [278-280] (Reviewed in [281]) |