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Global cerebral ischemia in rats leads to amnesia due to selective neuronal death followed by astroglial scar formation in the CA1 layer

Kevin Sadelli [a], Jean-Claude Stamegna [a], Stéphane D. Girard [a,1], Nathalie Baril [b], Guy Escoffier [a], Maïna Brus [a], Antoine D. Véron [a,c], Michel Khrestchatisky [a], François S. Roman [a,*]

[a] Aix Marseille Univ, CNRS, NICN, Marseille, France
[b] Aix Marseille Univ, Fédération de recherche 3C, FR 3512, Marseille, France
[c] IRSEA, Research Institute in Semiochemistry and Applied Ethology, 84400 Apt, France

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ABSTRACT

Global Cerebral Ischemia (GCI) occurs following cardiac arrest or neonatal asphyxia and leads to harmful neurological consequences. In most cases, patients who survive cardiac arrest develop severe cognitive and motor impairments. This study focused on learning and memory deficits associated with brain neuroanatomical reorganization that appears after GCI. The four-vessel occlusion (4VO) model was performed to produce a transient GCI. Hippocampal lesions in ischemic rats were visualized using anatomical Magnetic Resonance Imaging (aMRI). Then, the learning and memory abilities of control and ischemic (bilaterally or unilaterally) rats were assessed through the olfactory associated learning task. Finally, a "longitudinal" histological study was carried out to highlight the cellular reorganizations occurring after GCI. We demonstrated that the imaging, behavioral and histological results are closely related. In fact, aMRI revealed the appearance of hyper-intense signals in the dorsal hippocampus at day 3 post-GCI. Consequently, we showed a rise in cell proliferation (Ki 67$^+$ cells) and endogenous neurogenesis especially in the dentate gyrus (DG) at day 3 post-GCI. Then, hyper-intense signals in the dorsal hippocampus were confirmed by strong neuronal losses in the CA1 layer at day 7 post-GCI. These results were linked with severe learning and memory impairments only in bilaterally ischemic rats at day 14 post-GCI. This amnesia was accompanied by huge astroglial and microglial hyperactivity at day 30 post-GCI. Finally, Nestin$^+$ cells and astrocytes gave rise to astroglial scars, which persisted 60 days post-GCI. In the light of these results, the 4VO model appears a reliable method to produce amnesia in order to study and develop new therapeutic strategies.

1. Introduction

The central nervous system (CNS) is a complex entity subject to various neurological disorders. Different forms of brain lesions can occur during life such as stroke, trauma or neurodegenerative diseases. These disorders are all characterized by the loss of nerve cells, cognitive impairments and/or sensorimotor dysfunctions. According to the World Health Organization (WHO), the burden of brain disorders constitutes 35–38% of the total burden of all diseases and about 1.5 billion people worldwide suffer from CNS disorders (Pardridge, 2003). The most common CNS pathology is stroke, which represents the second leading cause of death worldwide. Cardiac arrest, asphyxia, shock, serious hypertension, brain injuries, and surgery related to the heart and thorax can cause temporary GCI, a stroke sub-type (Madl & Holzer, 2004). The brain has limited tolerance to ischemia in addition to being one of the most vulnerable organs to ischemia due to its restricted anaerobic metabolism and glycogen stores. Various models of cerebral ischemia have been developed and described in recent decades (Traystman, 2003), and each of them has advantages and

Abbreviations: GCI, Global Cerebral Ischemia; 4VO, four-vessel occlusion; CNS, central nervous system; CCA, common carotid arteries; VA, vertebral arteries; MRI, magnetic resonance imaging; MRA, magnetic resonance angiography; MIP, maximum intensity projection; ICA, internal carotid artery; BA, basilar artery; UNI, unilaterally; BI, bilaterally; NeuN, neuronal nuclei; GFAP, glial fibrillary acidic protein; DCX, doublecortin; Iba1, Ionized calcium Binding Adaptor molecule 1; CA, ammonic field; DG, dentate gyrus; SR, stratum radiatum; rt-PA, tissular Plasminogen Activator.

* Corresponding author at: Neurobiology of Mnesic Processes, Centre St Charles, 3 Place Victor Hugo Case B, 13331 Marseille Cedex 03, France.
E-mail address: francois.roman@univ-amu.fr (F.S. Roman).
[1] Present address: Vect-Horus S.A.S., Faculté de Médecine Secteur Nord, CS80011, Boulevard Pierre Dramard, 13344 Marseille Cedex 15, France.

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limitations (Dirnagl, Iadecola, & Moskowitz, 1999). Two main types of cerebral ischemia models have been proposed: focal and global.

GCI models do not mimic an ischemic stroke condition as defined by the WHO. Instead, they mimic the neurological consequences that occur as a result of cardiac arrest. These models have the potential to be clinically informative considering that about 50% of all patients who survive cardiac arrest develop persistent and severe cognition deficits and motor function impairments (Lim, Alexander, LaFleche, Schnyer, & Verfaellie, 2004). Many methods are available to produce GCI such as decapitation (Lowry, Passonneau, Hasselberger, & Schulz, 1964), strangulation (Siemkowicz & Gjedde, 1980), ventricular fibrillation (Berkowitz et al., 1991), the two-vessel (Eklof & Siesjo, 1972) and the 4VO (Pulsinelli & Brierley, 1979) models.

The 4VO model was favored due to its potential to damage different brain structures depending on ischemia duration. In rats, this model was used to mimic cardiac arrest, which was followed by bilateral, global and transient ischemia of the forebrain like in humans (Pulsinelli & Brierley, 1979). In particular, the CA1 pyramidal neurons of the dorsal hippocampus are more significantly affected by temporary and short-term ischemia compared to other regions of the brain (Kirino, Tamura, & Sano, 1985). The hippocampal CA1 pyramidal cells selectively die after GCI (Schmidt-Kastner & Freund, 1991). It is well known that the hippocampus is principally involved in learning and declarative long-term memory (Marchetti, Dumuis, Bockaert, Soumireu-Mourat, & Roman, 2000). The hippocampal formation is a functional segment of the limbic system, and impairment in this area may also result in the evolution of neuropsychiatric symptoms such as amnesia.

Neurobehavioral tests performed to assess precisely learning and memory impairments due to GCI are only based on spatial learning and memory tasks (Quintard et al., 2011; Wan et al., 2015). In this study, we proposed to evaluate the 4VO model in the olfactory associative learning task (Roman, Staubli, & Lynch, 1987), which distinguishes different types of memory. The specific learning and memory deficits were evaluated with regard to human amnesia following GCI. In fact, damage to hippocampal and retrohippocampal structures in rodents, like in humans, causes selective impairments in a variety of spatial and non-spatial declarative-like learning tasks (Scoville & Milner, 1957; Hirsh, 1974; Squire & Zola, 1996; Jeltsch, Bertrand, Lazarus, & Cassel, 2001). More recently, it has been shown that the dorso-lateral striatum and the dorsal hippocampus contribute to successful learning and memory in rats from procedural to declarative-like memory (Jacquet et al., 2013).

The aim of this study was to evaluate the ability of the 4VO model to produce a reliable method for amnesia, as close as possible to that observed in humans. MRI acquisitions were performed to predict hippocampal lesions and then the olfactory associative learning task was used to assess the learning and memory abilities of control and ischemic rats. Finally, immunolabeling was carried out to highlight the cellular reorganization occurring following the 4VO model.

2. Materials & methods

2.1. Animals

Two-month-old male Sprague Dawley (OFA) rats (n = 40; Charles River) were used. All animals were housed in individual cages and maintained in a 12-h light/12-h dark cycle at a constant temperature (21 ± 1 °C). Food and water were provided ad libitum except during the behavioral assessment. Anesthesia and surgical procedures were performed according to the European regulation on Animal Care Guidelines, and our protocols were approved by the Animal Care Committee of Aix-Marseille University (accreditation No. C13-055-6 of the French Ministry of Agriculture).

2.2. Protocol design

Of the forty rats used in our study (Fig. 1A), twenty-four were subjected to GCI. The 4VO model was used according to a two-step procedure over two days (day −1 and 0) as previously described (Pulsinelli & Brierley, 1979). Immediately at the end of the thermo-cauterization surgical procedure (day −1), magnetic resonance angiography (MRA) acquisitions were performed on all rats to follow the time course of vascular occlusion and reopening. At days 1, 3 and 7, anatomical magnetic resonance imaging (aMRI) was assessed in twelve rats (8 ischemic and 4 control rats) to reveal the extent of lesions. Then, animals were arbitrarily shared between two groups and sacrificed at day 3 or 7. At fourteen days post-GCI, the learning and memory abilities of control (n = 12) and ischemic rats (n = 16) were assessed using a hippocampal-dependent behavioral task based on olfactory associative discrimination. Finally, at 30 or 60 days post-GCI, all rats were perfused to compare some histological features between control and ischemic rats.

2.3. GCI induction by the 4VO model

Step 1: permanent thermo-cauterization of vertebral arteries (VA)

Rats weighing between 250 and 300 g were anesthetized using a mixture of air (2 L/min) and isoflurane, 3% for induction into a hermetic cage and 2% for maintenance. General (Buprenorphine, 0.03 mg/kg i.p.) and local analgesia (Lidocaine, subcutaneous) were performed. Then, as described previously (Pulsinelli & Brierley, 1979), a 0.2 mm diameter electrocautery needle was inserted through each alar foramen and both VA were thermo-cauterized.

Step 2: occlusion of bilateral and transient common carotid arteries (CCA)

The next day, under the same anesthesia conditions described above, the surgical field was prepared. Then, gaseous anesthesia was interrupted, the CCA were isolated from nerves and occluded using microvascular clamps (FST #00400-03) to produce the complete 4VO. During vascular occlusion, body temperature was monitored and/or maintained at 38 °C ± 0.5 with a rectal thermostat coupled to an electric blanket. Animals were kept in the recumbent position to yield the maximal stimulus for the righting reflex. Their level of consciousness, the presence or absence of a corneal reflex, their ability to walk and to climb was observed. Animals that reacted following previous stimuli were excluded from the study. Fifteen minutes later, microvascular clamps were removed to stop the occlusion and enable restoration of the carotid blood flow.

2.4. In vivo magnetic resonance imaging (MRI) acquisitions

All experiments were performed on a 70/16 PharmaScan spectrometer (Bruker Biospin, Ettlingen, Germany) equipped with a 7-Tesla magnet and 16-cm horizontal bore size. A linear birdcage coil with a 38-mm inner diameter was used for signal transmission and reception. In all experiments, rats were maintained under isoflurane anesthesia via the nose-cone of a rat head-holder device.

MRA started immediately following the end of the thermo-cauterization surgical procedure (day −1) using a two-dimensional FLASH-TOF sequence technique (TE = 4 ms, TR = 12 ms, FA = 60 °, slice thickness = 0.4 mm, FOV = 32 × 32 mm^2, matrix = 256 × 256, 3 averages). Angiograms were achieved by