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Research report

Ischemic lesions localized to the medial prefrontal cortex produce selective deficits in measures of executive function in rats

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HIGHLIGHTS

- There are few pre-clinical models for higher-order cognitive dysfunction following stroke.
- Microinjections of endothelin-1 result in ischemic lesions in the medial prefrontal cortex.
- Lesions result in select, but not generalized, deficits in a set-shifting task.
- Lesions do not significantly affect temporal order memory.

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ABSTRACT

Ischemic stroke is one of the leading causes of neurological disability worldwide, and it has been estimated that about one quarter of stroke survivors experience some measurable long-term cognitive impairments. Many higher order cognitive deficits occur because of damage to the prefrontal cortex (PFC), which is one of the main areas of the brain responsible for executive functioning in mammals. Currently, there are few animal models that examine the effects of stroke on executive function. In this study we used bilateral micro-injections (1 μl) of the vasoconstricting peptide endothelin-1 (ET-1) into the medial PFC in male Sprague-Dawley rats (or vehicle control, $N = 17–18$ per group) in order to model ischemic lesions in the medial PFC. The effects of these lesions on executive function were assessed using tests of set-shifting and temporal object recognition. ET-1 injections in the medial PFC resulted in replicable and specific lesions within the PFC with an average infarct volume of 16.63 ± 2.71 mm^3. The ischemic lesions resulted in specific contextual set-shifting deficits within the maze, including an increased number of trials to criterion and a significant difference in learning curves. However, no deficits in temporal order memory processing were noted between sham and stroke animals. We conclude that ischemic lesions localized to the mPFC result in selective but not generalized deficits in executive function in rats.

1. Introduction

Stroke is one of the leading causes of death and disability, with an estimated 795,000 new or recurring cases occurring each year in the United States alone [1]. Although a majority of patients survive the initial cerebrovascular insult, many affected individuals have ongoing deficits in function which may persist for months or years post-stroke [2]. The deficits induced by stroke damage can be broadly classified into two general categories: motor deficits and cognitive deficits. Although new methods have been, and continue to be developed to treat post-stroke motor deficits, there are few demonstrably effective strategies to treat the many forms of cognitive dysfunction that may occur post-stroke [3,4]. As well, patients suffering from cognitive deficits are less receptive to therapies used to treat accompanying motor dysfunctions, exacerbating the effects of stroke and hindering recovery [5,6]. Many types of post-stroke cognitive deficits have been described, including encoding-based deficits, aphasia, and executive dysfunction. Deficits in executive functions, which are considered higher order cognitive functions controlled by the prefrontal cortex, include working memory, goal-based decision making, and the learning and proper application of rules [7,8]. In patients who have survived an initial stroke, it has

Abbreviations: aCSF, artificial cerebral spinal fluid; SST, set-shifting task; ET-1, endothelin-1; PFC, prefrontal cortex; PSD, post-surgery day.

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been estimated that approximately one half have some measureable long-term deficit in executive function [9].

Although the mechanisms by which restricted blood flow causes damage to the brain are relatively well understood, the potential deficits in behaviour resulting from this damage as well as treatment strategies to alleviate this damage are less understood and elusive, respectively, and further understanding relies heavily on animal models [10,11]. One of the most commonly used stroke model, the rat middle cerebral artery occlusion (MCAo) model, produces a replicable and reliable lesion both in the cortex and striatum, and results in both motor and some cognitive deficits [12,13]. Unfortunately because of the size and location of the lesion, which is typically centred within the occipital lobe, the study of complex cognitive deficits in rats is confounded by overlapping sensory and motor deficits and the lack of documented executive function deficits [14,15]. Other commonly used models such as 2- and 4-vessel occlusion and hypoxia-ischemia are similarly compromised in that the lesions are typically localized to specific regions, notably the CA1 region of the hippocampus, and photothrombotic models are largely restricted to surface vasculature [16,17]. Recently, we have described how small well-localized ischemic lesions produced by surgical microinjection of endothelin-1 (ET-1) have been used successfully to produce discreet functional deficits in rats [18–20]. Endothelin-1 is a potent vasoconstrictor capable of temporarily occluding blood vessels via an action on endothelin receptors [21]. This feature of temporary occlusion allows ET-1 injections to mimic the effects of a temporary ischemic stroke. The compound can be injected directly onto blood vessels in the brain in order to mimic already established surgical protocols, or it can be injected within the brain itself, resulting in small vessel occlusion leading to the development of an ischemic lesion directly at the site of application or injection [22,23].

Non-ischemic lesions that occur within the prefrontal cortex (PFC) of rats can cause a variety of different behavioral effects, including temporal order memory dysfunction, working memory dysfunction, an attenuated fear response, alterations in social interaction, and deficits in attentional processing [24–27]. In humans, ischemic lesions in the dorso-lateral PFC, which is functionally equivalent to the medial PFC region in rats, cause a variety of functional deficits including an increased incidence in depressive symptoms and attentional set-shifting difficulties [28,29].

The objectives of this study were two-fold: first, to localize a stroke-induced lesion to the medial prefrontal cortex via the injection of the vasoconstrictor ET-1, and second to evaluate any resulting deficits in executive function using a temporal order memory task and a set-shifting task.

2. Methods

2.1. Experimental animals

All procedures were conducted in accordance with the guidelines of the Canadian Council for Animal Care and were approved in advance by the University of Prince Edward Island Animal Care Committee. Adult male Sprague-Dawley rats ($n = 37$, 250–275 g on arrival) were purchased from Charles River Laboratories (Montreal, Canada) and singly housed on a reverse 12 h light/dark cycle (lights on at 18:00 and off at 06:00) with food (Purina rat chow) and water available ad libitum until training/testing (see below). Upon arrival, animals were acclimated to the facility for one week and were handled by the experimenter for 5 min each day for three days subsequent to the acclimation period. Subsequent to daily handling all animals were given 10 sucrose pellets (Bio-Serv, Frenchtown, USA) in their home cage in order to acclimate the animals to the reward. All behavioral training and testing occurred during the dark phase of the light cycle.

2.2. Surgical procedures

The surgical protocols for producing an ET-1 stroke lesion were as reported previously with the exception of injection coordinates and volumes [18,19]. Rats (ET-1 $n = 19$, sham $n = 18$) were anaesthetised by being placed into an induction box prefilled with 3.5% isoflurane (PPC, Richmond Hill, Canada) in oxygen for 8 min, and anaesthesia was maintained throughout the surgery with a 2–3% isoflurane mixture. Animals were mounted on a stereotaxic frame (Kopf Instruments, Tujunga, USA), and the heads were shaved. Once shaved, topical Xylocaine (AstraZeneca, Mississauga, Canada) was applied to the shaved area and left for 5 min before a subsequent 2 cm midline incision was made down the top of the cranium. This incision was held open by 4 clamps. Two small burr holes were then drilled into the cranium above the coordinates for drug injection. A 26 gauge 10 μl syringe (Hamilton, Reno, USA) was lowered into each of the injection sites (anterior/posterior +3.0, medial/lateral ±0.7, dorsal/ventral −4.5, all coordinates relative to bregma) and was left for 1 min prior to ET-1 injection. After one minute, 1 μl of ET-1 (400 pmol) dissolved in artificial cerebral spinal fluid (aCSF) was injected into the cortex at a rate of 0.5 μl/min. Once injected, the needle was left undisturbed for 4 min to allow for drug dispersal at the injection site, and then the needle was slowly retracted from the brain. After both ET-1 injections, the incision site was sutured and xylocaine was reapplied. At the conclusion of the surgery each animal was given a 2.0 mg/kg subcutaneous injection of butorphanol (Wyeth, Guelph, Ontario) for post-operative analgesia. Sham treated animals received the same surgical treatment as stroke animals but received an equal volume injection of aCSF.

2.3. Set-shifting task (SST)

All animals were habituated and trained in the set-shift maze over a two week period prior to surgery (Section 2.2). The maze and testing protocol was modified from Stefani et al. [32]. The apparatus was a plus-maze constructed of Plexiglas walls (20 cm high) with metal arms (40 × 10.5 cm length and width). Each arm was lined with a Plexiglas insert which varied along two different stimulus dimensions: brightness or texture. The inserts were painted either black or white, with one arm of each colour painted with the inclusion of bird gravel (Hagen, Montreal, Canada) to create a rough texture. Each of the inserts spanned the full length of the arm, and the centre rectangle of the maze consisted of a 11.5 × 10.5 cm Plexiglas insert, painted grey (see Fig. 1). The day prior to any training or testing, the animal was placed on a restricted feeding schedule, which consisted of 4 h of ad libitum feeding followed by 20 h of food deprivation. Once 20 h had passed, the training or testing commenced and the animal was placed back on ad libitum feeding once the training or testing was complete.

2.3.1. SST training protocol

The training regimen prior to surgery was divided into two separate sections conducted over two weeks. The first week of training consisted of placing the animal in the maze with sucrose pellets in all of the arms and in each of the food cups located at the ends of the arms. The animals remained in the maze for 5 min or until each of the food pellets was consumed. Over the course of the first week the number of food pellets in the maze was reduced until the last day when there was only 1 pellet in each of the food cups.

The second week of training consisted of placing the animal pseudo-randomly into one of the four arms of the maze, and blocking access to the arm directly opposite the start arm, thereby creating a T-maze. The food cups of the right and left maze arms