Behavioural Brain Research 219 (2011) 227–233

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Impaired conditioned emotional response and object recognition are concomitant to neuronal damage in the amygdala and perirhinal cortex in middle-aged ischemic rats

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ARTICLE INFO

Article history:
Received 1 October 2010
Received in revised form
22 December 2010
Accepted 10 January 2011
Available online 14 January 2011

Keywords:
Conditioned emotional response
Fear
Basolateral amygdala
Perirhinal cortex
Forebrain ischemia

ABSTRACT

The current study characterizes fear conditioning responses following global ischemia and evaluates neuronal damage affecting discrete extra-hippocampal areas susceptible to contribute to post ischemic emotional and memory impairments. Conditioned emotional response, Barnes Maze and object recognition tests were used to assess emotional, spatial and recognition memory, respectively. Behavioural testing was initiated in middle-aged animals (10–12 month old) 1 week following sham ($n = 16$) or 4VO occlusion ($n = 18$). Post-mortem cellular assessment was performed in the hippocampal CA1 layer, the perirhinal cortex and basolateral amygdala. Middle-aged ischemic animals showed impaired spatial memory in the initial three testing days in the Barnes Maze and deficit in recognition memory. Of interest, ischemic rats demonstrated a significant reduction of freezing and increased locomotion during the contextual fear testing period, suggesting reduced fear in these animals. Assessment of neuronal density 40 days following global ischemia revealed that CA1 neuronal injury was accompanied by 20–25% neuronal loss in the basolateral nucleus of the amygdala and perirhinal cortex in middle-aged ischemic compared to sham-operated animals. This study represents the first demonstration of altered conditioned fear responses following ischemia. Our findings also indicate a vulnerability of extra-hippocampal neurons to ischemic injury, possibly contributing to discrete emotional and/or memory impairments post ischemia.

1. Introduction

Global ischemia, which reduction of cerebral blood flow mimics that of a cardiac arrest, leads to neuronal death accompanied by cognitive impairments in rats and humans [31,32,42,49,59]. Past studies have mainly focused on the cognitive deficits associated with damage of the hippocampal CA1 neurons which are known to be essential to spatial memory [29]. These long-lasting spatial impairments have been shown in navigation tests such as the Morris Water Maze [8–10] or land mazes like the radial or Barnes Maze [54,84]. However, non spatial memory impairments and alterations of emotional reactivity reported in ischemic animals suggest the involvement of extrahippocampal neuronal damage. Thus, object recognition deficit is observed following perirhinal cortex lesions in rats, while lesions to the hippocampal formation leaves anterograde recognition memory intact [3,14,51]. In addition, reports of comparable post ischemic hyperactivity in preconditioned ischemic rats and gerbils despite significant CA1 neuronal protection [18,61] suggest that mediation of this behavioural response involves neurochemical changes and/or brain damage affecting other brain regions. In this context, disinhibition to explore unfamiliar and/or mildly anxiogenic environments and differential behavioural/emotional reactivity have been shown to contribute to heightened open field exploration in ischemic animals [47,48,62].

Recent findings have demonstrated ischemia-induced alterations of cellular activity in brain regions outside the CA1 region, which could have a significant impact on behavioural recovery following ischemia. Thus, Caruana et al. [14] reported epileptiform EEG activity in the CA1/subicular region, the perirhinal cortex, and the prefrontal cortex following 15 min global ischemia in rats lasting several hours post reperfusion. Moreover, increased amygdalar CRH mRNA expression and release has been demonstrated following global and focal ischemia in rats [37,82], while significant losses of CRH-positive neurons have recently been demonstrated in the same brain region 6 weeks following hypoxia–ischemia [13], a phenomenon associated with hyperactivity in response to novel open field exposure in ischemic rats. Reduced amygdalar

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doi:10.1016/j.bbr.2011.01.009

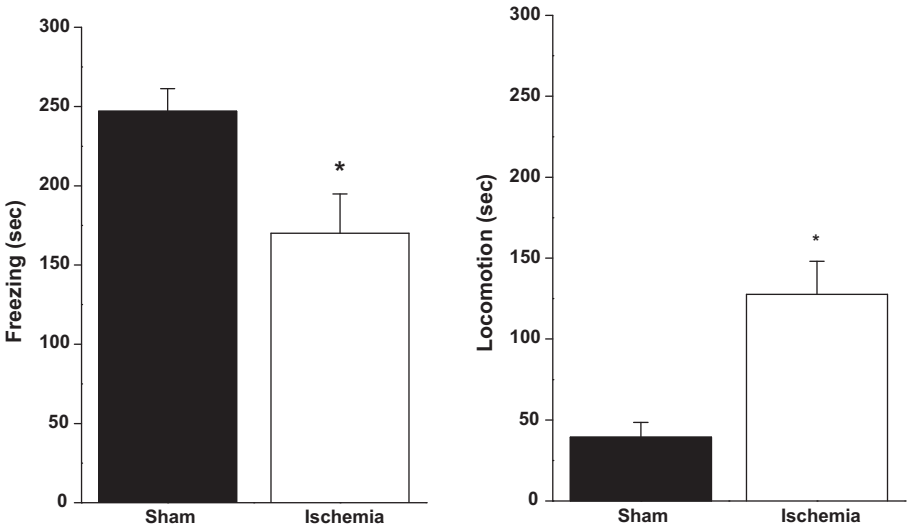

Fig. 1. Time engaged in freezing behaviour and locomotion in ischemic and sham-operated rats during the 5 min contextual fear conditioning test. Ischemic rats showed significantly less freezing ($p = 0.014$) and a significant increase in locomotor activity ($p = 0.000$) as compared to sham animals. Values are expressed as mean ± S.E.M.

volume in stroke and cardiac arrest survivors has also been correlated with cognitive impairments [27,72]. The role of the amygdala in emotional learning and conditioned emotional responses is well acknowledged [22,23,35,36,46] and lesion of the basolateral amygdala shown to result in extinction or significant inhibition of contextual conditioned fear translated by more agitation and less freezing [43,44].

To date, no studies have assessed conditioned emotional responses following cerebral ischemia although changes in emotional reactivity and anxiety have been shown in ischemic animals [47,62,83]. As highlighted above, different studies recently indicated alterations of neuronal activity in brain regions outside the CA1 area known to be associated with emotional regulation and recognition memory post ischemia. Thus, the goals of the current study are (1) to characterize conditioned emotional responses following global ischemia in rats and (2) to evaluate the possibility that behavioural deficits observed in ischemic animals be accompanied by extra-hippocampal brain damage affecting the perirhinal cortex and/or basolateral amygdala. The novel object preference, Barnes Maze and fear conditioning tests will be used to measure anterograde object recognition, spatial and emotional memory, respectively. Although stroke prevalence increases with age, behavioural and neuronal assessments post-ischemia remain mostly performed in young animals. This study using 10–12 month old rats will help to compare behavioural and neuronal changes observed in young and older ischemic animals.

2. Materials and methods

2.1. Subjects

Male Wistar rats ($N = 34$) aged 10–12 months, weighing between 550 and 900 g at time of arrival in our facility, were obtained from the Harlan Laboratory (Rochester, NY, USA). They were individually housed and maintained on a 12 h light/dark cycle (lights on at 7:00 a.m.) with free access to water and standard rat chow. The room temperature was maintained at 21–23 °C with 60% relative humidity. Upon arrival, animals were acclimatized to the animal facility for at least 1 week prior to surgery. All procedures were carried out in accordance with the guidelines set by the Canadian Council of Animal Care and were approved by the University of Ottawa Animal Care Committee. The experimental protocol included one group of rats submitted to forebrain ischemia ($n = 18$) and one control group receiving a sham surgery ($n = 16$). All rats had free access to ad libitum food and water during the entire post-ischemic reperfusion period.

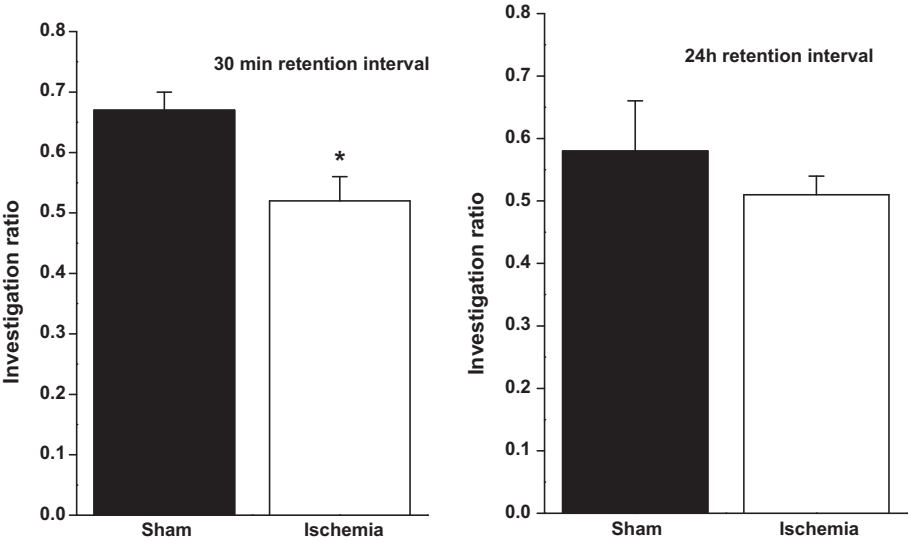

Fig. 2. Mean investigation ratio of ischemic and sham animals in the object recognition test at the 30 min and 24 h retention intervals. The asterisk indicates significant reduction of the exploration ratio following 10 min global ischemia ($p < 0.05$). Values represent mean investigation ratio ± S.E.M.