Behavioural Brain Research 316 (2017) 29–37

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Alterations in anxiety and social behaviour in Npas4 deficient mice following photochemically-induced focal cortical stroke

T.S. Klarić[a,1], E.J. Jaehne[b,1], S.A. Koblar[a], B.T. Baune[b], M.D Lewis[c,*]

[a] School of Medicine, University of Adelaide, North Terrace, Adelaide, SA, 5005, Australia
[b] Discipline of Psychiatry, University of Adelaide, North Terrace, Adelaide, SA, 5005, Australia
[c] South Australian Health & Medical Research Institute, North Terrace, Adelaide, SA, 5000, Australia

HIGHLIGHTS

- Wildtype and Npas4 knockout mice were compared in behavioural and cognitive tests following photochemically-induced stroke.
- Spatial recognition memory is impaired in Npas4$^{-/-}$ and wildtype mice after stroke.
- Despair-like behaviour is reduced in both Npas4$^{-/-}$ and wildtype mice after stroke.
- Stroke leads to increased anxiety in Npas4$^{-/-}$ mice but not wildtype mice.
- Stroke leads to decreased sociability in wildtype mice but not in Npas4$^{-/-}$ mice.

ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form 21 August 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
Npas4
Photochemical stroke
Anxiety
Sociability
Behaviour

ABSTRACT

In addition to causing widespread cell death and loss of brain function, cerebral ischaemia also induces extensive neuroplasticity. In humans, stroke is often accompanied by severe cognitive and psychiatric changes that are thought to arise as a consequence of this infarct-induced remodelling. A candidate for producing these post-stroke neuropsychiatric changes is Npas4, an activity-dependent transcription factor involved in synaptic plasticity whose expression is aberrantly up-regulated following ischaemic injury. In this study we investigated the role of Npas4 in modulating these stroke-induced neuropsychiatric responses by comparing the performance of wildtype and Npas4$^{-/-}$ mice in various cognitive and behavioural tasks in a photochemical model of focal cortical stroke. We show that this stroke model results in impaired spatial recognition memory and a reduction in despair-like behaviour that affect both genotypes to a similar degree. Moreover, mice lacking Npas4 also show differences in some aspects of post-stroke sociability and anxiety. Specifically, we show that while stroke had no effect on anxiety levels in wildtype mice, Npas4$^{-/-}$ mice became significantly more anxious following stroke. In addition, Npas4$^{-/-}$ mice retained a greater level of sociability in the acute post-stroke period in comparison to their wildtype littermates. Thus, our findings suggest that Npas4 may be involved in post-stroke psychiatric changes related to anxiety and sociability.

1. Introduction

Stroke is the second leading cause of death and the third most common cause of disability worldwide [1]. Cerebral ischemia initiates a complex and dynamic series of cellular and molecular events that ultimately lead to neuronal death and loss of brain function [2]. It is well known that ischaemic damage induces widespread neuroplasticity throughout the brain which in turn leads to extensive structural reorganisation [3–5]. This neurophysiological remodelling is thought to be an adaptive process that promotes recovery of motor function. However, it may also be relevant to the various

Abbreviations: ANOVA, analysis of variance; Bdnf, brain-derived neurotrophic factor; CUMS, chronic unpredictable mild stress; GABA, γ-aminobutyric acid; FST, forced swim test; MCAo, middle cerebral artery occlusion; Npas4, neuronal PAS domain-containing protein 4; OFT, open field test; PI, preference index; PSD, post-stroke depression; SEM, standard error of the mean; SPT, saccharin preference test.

* Corresponding author at: SAHMRI, PO Box 11060, Adelaide, SA, 5001, Australia.
E-mail address: martin.lewis@sahmri.com (M.D Lewis).

[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.bbr.2016.08.050
0166-4328/

behavioural and psychiatric changes that are often seen following stroke.

In addition to the debilitating paresis that is caused by impaired motor function, ischaemic injury can often produce profound changes in a person's affect, sociability and cognition. One of the most common neuropsychiatric conditions that accompanies cerebral infarction is post-stroke depression (PSD), which affects around 30% of stroke patients [6,7]. Patients affected by stroke can also present with anxiety [8,9], serious cognitive impairment [10] and social deficits [11]. These psychiatric complications hamper recovery and further reduce quality of life [12] which is why the combination of physical and psychiatric comorbidities makes stroke a particularly devastating condition. To date, little is known about the underlying molecular factors that contribute to altered mood and cognition following stroke. A better understanding of the basic neurobiology involved in these processes may enable us to provide improved therapeutic treatments for patients affected by stroke-related psychiatric conditions. Accordingly, identification of the key players involved in these processes remains a priority.

One candidate is neuronal PAS domain-containing protein 4 (Npas4), an activity-dependent transcription factor with roles in synaptic plasticity whose expression is radically up-regulated following ischaemic brain injury in animal models of stroke [13–15]. Npas4 plays an important role in excitatory/inhibitory homeostasis in neural networks by regulating the formation of inhibitory synapses on excitatory neurons [16,17]. It does so by driving a genetic programme that is responsible for effecting changes at synapses via activation of a number of plasticity-related genes, such as brain-derived neurotrophic factor (Bdnf) [17,18] and the actin-binding protein named developmentally regulated brain protein (Drebrin) [19]. There is growing evidence that dysregulation of Npas4 may be implicated in some types of psychiatric disorders, such as anxiety and depression [20–22]. In addition, there have been a number of recent studies that have highlighted the importance of Npas4 in learning and memory [23–25]. Nevertheless, while it has been demonstrated that Npas4 is critically linked to aspects of behaviour and cognition, it is not yet known whether the aberrant expression of Npas4 that is seen after stroke affects these processes.

Numerous rodent studies have described the acute changes in Npas4 expression that occur in response to ischaemic injury. A microarray study of post-stroke gene expression in the rat brain revealed that *Npas4* was one of the earliest and most profoundly up-regulated genes following two hours of middle cerebral artery occlusion (MCAo) [13,14]. Analysis of the spatial expression pattern of the *Npas4* transcript showed that, at the level of the infarct core, *Npas4* expression was elevated throughout the entire affected hemisphere, with no change in the contralateral hemisphere [14]. At the protein level, neuron-specific up-regulation of Npas4 was seen throughout the corticolimbic system of the ipsilateral hemisphere in regions associated with cognition and emotion, such as the frontal cortex, basal ganglia and amygdala [15]. This up-regulation is transient with *Npas4* transcript and protein expression returning to baseline levels within approximately 12 h of the onset of ischaemia [14,15]. Importantly for this study, we have previously demonstrated that photochemically-induced focal cortical ischaemia also results in extensive up-regulation of Npas4 expression in mice [26]. In this particular type of stroke model, which produces a roughly spherical lesion that affects all of the cortical layers, Npas4 protein expression is induced throughout the ipsilateral hemisphere but not the contralateral hemisphere or the lesion site itself. Nevertheless, though the pattern of *Npas4* expression after stroke has been studied in some detail, the physiological relevance of this aberrant up-regulation has yet to be determined.

When taken together, the evidence presented in the literature raises the possibility that Npas4 may play a role in the neuropsychiatric changes that accompany ischaemic brain injury. Due to its central role in neuronal connectivity and its aberrant expression pattern following cerebral ischaemia, Npas4 is a good candidate for linking stroke-induced molecular changes in nerve cells to altered behavioural and cognitive states. Based on this premise, we hypothesised that Npas4 may be involved in the lesion-induced plasticity that occurs following ischaemic injury and that mice lacking Npas4 would therefore perform differently in various cognitive and behavioural tasks after a stroke event due to their inability to activate Npas4-mediated plasticity. If aberrant Npas4 up-regulation is the cause of post-stroke neuropsychiatric changes, then one would expect that these adverse consequences would be alleviated in Npas4$^{-/-}$ mice. If, on the other hand, Npas4 expression protects against post-stroke behavioural and cognitive changes, then one would expect that these detrimental neuropsychiatric changes would be exacerbated in Npas4$^{-/-}$ mice. To address this question, we used a photochemical model of stroke to induce focal cortical ischaemia in both Npas4$^{+/+}$ and Npas4$^{-/-}$ mice and then compared their performance in various cognitive and behavioural tests.

2. Materials and methods

2.1. Mice

The transgenic mouse Npas4^{tm1Meg} (MGI:3828099), established on a C57BL/6J background was a gift from Prof. Yingxi Lin [17]. Heterozygotes were crossed to produce Npas4$^{-/-}$ and Npas4$^{+/+}$littermates. A total of 47 mice were used for surgery and behavioural testing. Equal numbers of male and female mice were used in each group. No statistically significance differences were found between genders in any of the tests (see Supplementary material). The ages of mice used for behavioural testing were; Npas4$^{+/+}$ Sham: 14.7 ± 0.33 weeks, Npas4$^{+/+}$ Stroke: 16.3 ± 1.9 weeks, Npas4$^{-/-}$ Sham: 11.0 ± 0.73 weeks and Npas4$^{-/-}$ Stroke: 15.5 ± 3.5 weeks. All experimental mice were housed in groups of 2–6 in individually ventilated cages. At all stages mice had food and water available *ad libitum* and ambient temperature of the housing and testing rooms was $22 \pm 1\,^{\circ}C$. Mice were housed under a 12hr light/dark cycle (lights on between 07:00-19:00 h) and all behavioural testing was conducted between 08:00 and 16:00 h. All animals were housed and treated in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The University of Adelaide Animal Ethics Committee approved all experiments prior to commencement (Animal Ethics #M-2011-58 and #M-2013-122).

2.2. Photochemical induction of focal cortical ischaemia

This procedure was performed as previously described [26]. Briefly, rose Bengal dye (Sigma-Aldrich, #R3877) was prepared fresh by dissolving in saline (10 mg/mL), filtering through a 0.45 μm filter (Minisart, #16555) and kept protected from light until use. Mice were intraperitoneally injected with Rose Bengal dye (100 mg/kg) prior to be being anaesthetised with inhaled isoflurane (Attane^{TM}, Bomac). The mice were then secured in a stereotaxic frame in which isoflurane was continuously delivered via a nose cone. An incision was made in the scalp and the skull exposed to reveal Bregma. The stereotaxic frame was then used to locate the target area, i.e. the region of the skull overlying the right forelimb motor cortex (1.5 mm lateral to Bregma, left hemisphere). A flexible light guide (Zeiss, #417075-9100000) attached to a light-emitting diode cold light source (Zeiss, CL 6000 LED, #435700-9101-000) was then positioned above the target area. The light source was fitted with a green light filter (Zeiss, #435700-9023-000) to restrict