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Research report

Hippocampal biomarkers of fear memory in an animal model of generalized anxiety disorder

Gisele Pereira Dias[a,b,c], Mário Cesar do Nascimento Bevilaqua[a,b,d], Anna Claudia Domingos Silveira da Luz[a,b], Renata Lopes Fleming[e], Lítia Alves de Carvalho[f], Graham Cocks[c], Danielle Beckman[a,e], Lucas Costa Hosken[a,b], William de Sant'Anna Machado[a,b], Ana Carolina Corrêa-e-Castro[a], Felippe Mousovich-Neto[g], Vítor de Castro Gomes[h,i], Gilmara de Nazareth Tavares Bastos[j], Regina Célia Cussa Kubrusly[l], Vânia Maria Corrêa da Costa[g], Deepak Srivastava[c], Jesus Landeira-Fernandez[h,m], Antonio Egidio Nardi[b], Sandrine Thuret[c,*], Patricia Franca Gardino[a]

[a] *Laboratory of Neurobiology of the Retina, Program of Neurobiology, Institute of Biophysics, Universidade Federal do Rio de Janeiro (UFRJ), Av Carlos Chagas Filho, 373, CCS, Bloco G, Sala C-031 – Cidade Universitária, CEP:21941-902 Rio de Janeiro, RJ, Brazil*[1]

[b] *Translational Neurobiology Unit, Laboratory of Panic & Respiration, Institute of Psychiatry, Universidade Federal do Rio de Janeiro (UFRJ), Avenida Venceslau Brás, 71 Fundos - Botafogo, CEP: 22290-140 Rio de Janeiro, RJ, Brazil*[2]

[c] *Institute of Psychiatry, King's College London, 125 Coldharbour Lane, London SE5 9NU, United Kingdom*

[d] *Health and Environment School, Universidade Castelo Branco, Av. de Santa Cruz, 1631 - Realengo, CEP: 21710-250 Rio de Janeiro, RJ, Brazil*

[e] *Laboratory of Neurochemistry, Program of Neurobiology, Institute of Biophysics, Universidade Federal do Rio de Janeiro (UFRJ), Av Carlos Chagas Filho, 373, CCS, Bloco G, Sala C-031 – Cidade Universitária, CEP: 21941-902 Rio de Janeiro, RJ, Brazil*[1]

[f] *Laboratory of Comparative and Developmental Neurobiology, Institute of Biophysics, Universidade Federal do Rio de Janeiro (UFRJ), Av Carlos Chagas Filho, 373, CCS, Bloco G – Cidade Universitária, CEP: 21941-902 Rio de Janeiro, RJ, Brazil*

[g] *Laboratory of Endocrine Physiology, Program of Physiology and Biophysics, Institute of Biophysics, Universidade Federal do Rio de Janeiro (UFRJ), Av Carlos Chagas Filho, 373, CCS, Bloco G – Cidade Universitária, CEP: 21941-902 Rio de Janeiro, RJ, Brazil*

[h] *Laboratory of Behavioral Neuroscience (LANEC), Department of Psychology, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), R. Marquês de São Vicente, 225 - Gávea, CEP: 22451-900 Rio de Janeiro, RJ, Brazil*

[i] *Department of Psychiatry and Neuroscience, Uniformed Services, University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD, USA*

[j] *Laboratory of Neuroinflammation, Institute of Biological Sciences, Universidade Federal do Pará (UFPA), Rua Augusto Corrêa 01, CEP: 66075-110 Belém, PA, Brazil*

[l] *Laboratory of Neuropharmacology, Department of Physiology and Pharmacology, Biomedics Institute, Universidade Federal Fluminense, Rua Prof. Hernani Pires de Melo 101/213 - São Domingos, CEP: 24210-130 Niterói, RJ, Brazil*

[m] *Laboratory of Comparative Psychology, Department of Psychology, Universidade Estácio de Sá (UNESA), R. do Bispo, 83 - Rio Comprido, CEP: 20261-060 Rio de Janeiro, RJ, Brazil*

HIGHLIGHTS

- Generalized Anxiety Disorder (GAD) is highly prevalent and incapacitating.
- The Carioca High-Conditioned Freezing (CHF) rats is a validated animal model for GAD.
- Besides enhanced anxiety, CHF rats present increased fear memory retention.
- Reduced neuroblast number and dendritic tree were found in the CHF dentate gyrus.
- Conversely, increased spine expression in the CHF dentate gyrus was also found.

* Corresponding author at: Department of Neuroscience, Centre for the Cellular Basis of Behaviour, James Black Centre, Institute of Psychiatry, King's College London, 125 Coldharbour Lane, SE5 9NU, London, United Kingdom. Tel.: +44 020 7848 5405; fax: +44 020 7848 5407.

E-mail addresses: giseledias@ufrj.br, gisele.dias@kcl.ac.uk (G.P. Dias), mariobevilaqua@gmail.com (M.C.d.N. Bevilaqua), annadomluz@gmail.com (A.C.D.S. da Luz), renataf@biof.ufrj.br (R.L. Fleming), litia.carvalho@gmail.com (L.A. de Carvalho), graham.d.cocks@kcl.ac.uk (G. Cocks), daniellebeckman@gmail.com (D. Beckman), lchosken@hotmail.com (L.C. Hosken), williamdesantannamachado@yahoo.com.br (W. de Sant'Anna Machado), anacarolina_castro@yahoo.com.br (A.C. Corrêa-e-Castro), felippeneto@hotmail.com (F. Mousovich-Neto), vitorcastrogomes@gmail.com (V. de Castro Gomes), gbastos@ufpa.br (G.d.N.T. Bastos), kubrusly@vm.uff.br (R.C.C. Kubrusly), vmccosta@biof.ufrj.br (V.M.C. da Costa), deepak.srivastava@kcl.ac.uk (D. Srivastava), landeira@puc-rio.br (J. Landeira-Fernandez), antonioenardi@gmail.com (A.E. Nardi), sandrine.1.thuret@kcl.ac.uk (S. Thuret), gardino@biof.ufrj.br (P.F. Gardino).

[1] INNT Translational Neuroscience (CNPq).

[2] INCT Translational Medicine (CNPq).

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ABSTRACT

Generalized anxiety disorder (GAD) is highly prevalent and incapacitating. Here we used the Carioca High-Conditioned Freezing (CHF) rats, a previously validated animal model for GAD, to identify biomarkers and structural changes in the hippocampus that could be part of the underlying mechanisms of their high-anxiety profile. Spatial and fear memory was assessed in the Morris water maze and passive avoidance test. Serum corticosterone levels, immunofluorescence for glucocorticoid receptors (GR) in the dentate gyrus (DG), and western blotting for hippocampal brain derived neurotrophic factor (BDNF) were performed. Immunohistochemistry for markers of cell proliferation (bromodeoxiuridine/Ki-67), neuroblasts (doublecortin), and cell survival were undertaken in the DG, along with spine staining (Golgi) and dendritic arborization tracing. Hippocampal GABA release was assessed by neurochemical assay.

Fear memory was higher among CHF rats whilst spatial learning was preserved. Serum corticosterone levels were increased, with decreased GR expression. No differences were observed in hippocampal cell proliferation/survival, but the number of newborn neurons was decreased, along with their number and length of tertiary dendrites. Increased expression of proBDNF and dendritic spines was observed; lower ratio of GABA release in the hippocampus was also verified. These findings suggest that generalized anxiety/fear could be associated with different hippocampal biomarkers, such as increased spine density, possibly as a compensatory mechanism for the decreased hippocampal number of neuroblasts and dendritic arborization triggered by high corticosterone. Disruption of GABAergic signaling and BDNF impairment are also proposed as part of the hippocampal mechanisms possibly underlying the anxious phenotype of this model.

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1. Introduction

Anxiety disorders, such as generalized anxiety disorder (GAD), are among the most prevalent psychiatric conditions [1]. GAD is characterized by generalized, chronic and excessive worry and anxiety, accompanied by disruptive somatic symptoms and cardiovascular changes [2]. It has been associated with significantly reduced quality of life [3], and it is estimated to be as incapacitating as depressive disorders [4]. Effective interventions require the investigation of the underlying structural and functional psychobiology of this condition, with appropriate animal models being a key tool for this.

As proposed for psychiatric disorders in general, GAD is a multifactorial condition, with disruptive functioning of different brain areas – such as the prefrontal and cingulate cortex, as well as the amygdala [5] – possibly participating together in its etiology and development. Furthermore, hippocampal abnormalities in GAD patients have also been proposed [6]. In rodents, some of the neurobiological aspects demonstrated to be involved in anxiety also include those related to hippocampal function and plasticity. In this context, hippocampal function can be modulated by higher levels of corticosterone [7], a neuroendocrine hallmark of the physiological stress usually associated with anxiety states [8]. In addition, elevated glucocorticoid levels have been related with reduced dendritic arborization in the hippocampus [9], an effect that can compromise successful synaptic transmission. Other important morphological indicators of the ability of a neuron to receive synaptic inputs and that can be altered in context of anxiety [10] are dendritic spines, micro-specializations of the dendritic shafts to establish functional contacts with other cells. Importantly, alterations in spine density are associated with changes in synaptic strength [11], especially in the context of excitatory synapses [12], which are crucial for spatial and fear memory formation [13–15]. Other parameters of hippocampal plasticity have also been shown to relate with anxious phenotypes. One of these parameters is the levels of brain-derived neurotrophic factor (BDNF) in the hippocampus [16], given its role in enhancing newborn cell survival, synaptic formation and plasticity [17]. Further to this, since the GABAergic system exerts a classic inhibitory effect, with its receptors being the target of widely used anxiolytic drugs [18], its disruption has also been associated with increased anxiety [19]. Finally, disturbance in the ability of the adult dentate gyrus (DG) of the hippocampus to continuously generate newly functional neurons, a process called adult hippocampal neurogenesis (AHN), has been shown to be associated with increased anxiety-related behaviors [20].

The hippocampus is a crucial structure for contextual fear learning, which is in turn considered an appropriate paradigm for establishing animal models of GAD [2,21,22], such as the Carioca High-Conditioned Freezing rats (CHF). A previous work established the CHF as a behaviorally validated model for the study of spontaneous high-anxiety [16], as differences were found only in anxiety-related paradigms and not in the forced swimming test of depression or the object recognition test, used as a paradigm for measuring cognitive skills. In order to further investigate potential factors comprising the biological basis of generalized anxiety, the present work analyzed hippocampal-dependent behavioral performance and biomarkers of hippocampal plasticity in the CHF rodents. We propose that generalized anxiety can result in part from multiple structural and biochemical plastic mechanisms in the hippocampus possibly orchestrated to protect the individual from chronic exposure to high corticosterone levels, but leading to facilitation of fear memory retention.

2. Materials and methods

2.1. Animals

Experimental procedures followed the Brazilian Society of Neuroscience and Behavior (SBNeC) guidelines, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications). Handling and methods of sacrifice were approved by the Committee for Animal Care and Use of the CCS/UFRJ (protocol # IBCCF002). Experimental animals (Carioca High-Freezing [CHF], a line of Wistar rats selectively bred for their high conditioned freezing response in contextual fear learning) were obtained as described previously [23]. Briefly, albino Wistar rats were exposed to the contextual fear conditioning paradigm, and selectively bred for differences in defensive freezing behavior in response to the conditioned context. Significant differences in freezing response were acquired after three generations of selective breeding (S3). In order to preserve the spontaneous differences between the experimental and the control groups, the animals used herein were not exposed to contextual fear conditioning or any aversive stimuli prior to experimentation. However, other individuals from all breeding generations were tested in

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