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Research report

Stress-induced hippocampus Npas4 mRNA expression relates to specific psychophysiological patterns of stress response

Jean-Baptiste Drouet [a], André Peinnequin [b], Patrice Faure [c], Josiane Denis [b], Nadine Fidier [a], Renaud Maury [a], Alain Buguet [d,e], Raymond Cespuglio [d,f], Frédéric Canini [a,g,*]

[a] Département Neurosciences & Contraintes Opérationnelles, Institut de Recherche Biomédicale des Armées (IRBA), BP73, 91223 Brétigny-sur-Orge, France
[b] Département des Services, Institut de Recherche Biomédicale des Armées (IRBA), BP73, 91223 Brétigny-sur-Orge, France
[c] Laboratoire d'étude de la physiopathologie de l'hypoxie (HP2), Inserm U 1042-Grenoble universités, Faculté de Médecine et de Pharmacie, Domaine de la Merci, 38700 La Tronche, France
[d] Faculté de Médecine, Centre de Recherche en Neurosciences de Lyon, Neurochem, 8 ave Rockefeller, F-69373 Lyon, France
[e] Malaria Research Unit, SMITh, ICBMS, UMR 5246 CNRS-INSA-CPE-University Lyon 1, Bâtiment Jules Raulin, 43 bvd du 11 Novembre 1918, 69100 Villeurbanne, France
[f] Guangzhou Medical University, Department of Anatomy, Xinzao Town-Panyu district, Guangzhou 511436, China
[g] Ecole du Val de Grâce, 1 place Laveran, F-75005 Paris, France

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ABSTRACT

Neuronal Per-Arnt-Sim (PAS) domain protein 4 (Npas4) is a key protein that intervenes in GABA synapse scaling and neurotrophicity enhancing. Since GABA and neurotrophicity are implicated in stress response and Npas4-deficient rodents exhibit behavioral alterations, an investigation was designed in rats to verify whether stress-induced spontaneous hippocampus Npas4 mRNA expression would be associated with specific patterns of stress response. The rats were exposed to one of three stressor levels: no stress (CTL, n = 15), exposure to a footshock apparatus (Sham, S, n = 40) and footshock (F, n = 80). After stress exposure the S and F rats were tested in an activity cage, and subsequently in an elevated plus maze (EPM), just prior to the sacrifice. Using cluster analysis, the animals already assigned to a stress level were also distributed into 2 subgroups depending on their Npas4 mRNA levels. The low (L) and high (H) Npas4 expression subgroups were identified in the S and F groups, the CTL group being independent of the Npas4 levels. The Npas4 effect was studied through the interaction between stress (S and F) and Npas4 level (L and H). The biological stress response was similar in H and L rats, except blood corticosterone that was slightly lower in the H rats. The H rats were more active in the actimetry cage and presented higher levels of exploration in the EPM. They also exhibited higher hippocampus activation, as assessed by the c-fos, Egr1 and Arc mRNA levels. Therefore high Npas4 expression favors stress management.

1. Introduction

Npas4 [neuronal Per-Arnt-Sim (PAS) domain protein 4], also known as Nxf (Ooe et al., 2004) or limbic-enriched (LE) PAS (Moser et al., 2004), is an activity-regulated transcription factor. It belongs to the basic helix-loop-helix-PAS (bHLH-PAS) protein family. Npas4 is an important target for regulating the reaction to stressors. Npas4 expression occurs very early in the activation process of neurons. Triggered by membrane depolarisation, Ca^{2+} influx (Lin et al., 2008) and cell stress (Ooe et al., 2009), Npas4 acts as a transcriptional enhancer (Benito and Barco, 2014; Kim et al., 2010), that models genomic activation. Npas4 is highly expressed in the brain and mainly in the frontal cortex, the entorhinal cortex and the hippocampus (Moser et al., 2004; Shamloo et al., 2006). Further, it is selectively expressed in neurons (Lin et al., 2008; Shamloo et al., 2006). Npas4 triggers the development of inhibitory GABA synapses on excitatory

Abbreviations: AdorA1, adenosine receptor A1; ANOVA, analysis of variance; Arbp, ribosomal protein large P0; AID, activity-regulated inhibitor of death; Arc, activity-regulated cytoskeleton; Bcl2, B-cell lymphoma 2; Bdnf, brain-derived neurotrophic factor; c-fos, FBJ osteosarcoma oncogene; CRF, corticotropin releasing factor; CTL, control; Egr1, early growth response 1; EPM, elevated plus maze; F, footshock; GABA, gamma amino butyric acid; H, high Npas4 level; HPA, hypotha lamo-pituitary-adrenocortical; Hprt, hypoxanthine phosphoribosyltransferase 1; Hsp-70, heat shock protein 70; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; L, low Npas4 level; Nf-κb1, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; NMDA, *N*-methyl-D-aspartate; Npas4, neuronal Per-Arnt-Sim (PAS) domain protein 4; Ppia, peptidylprolyl isomerase A (cyclophilin A); PTSD, post-traumatic stress disorder; Psd-95, postsynaptic density protein 95; S, sham; t-PA, plasminogen activator tissue; TrkB, tropomyosin receptor kinase B.

* Corresponding author at: Département Neurosciences & Contraintes Opérationnelles, Institut de recherche Biomédicale des Armées (IRBA), BP73, F-91223 Brétigny-sur-Orge, France.

E-mail address: frederic.canini@intradef.gouv.fr (F. Canini).

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pyramidal neurons of hippocampus, increasing the number of GABA synapses on the soma but reducing it in dendrites (Bloodgood et al., 2013). It results in the filtration of neuronal signals. The modulation of hippocampus GABA synapses is of special interest as GABA synapses are key structures in the hippocampus network functioning (Mann and Paulsen, 2007). They modulate anxiety (Engin and Treit, 2007), attention (Bast et al., 2017), sensorimotor integration (McGarrity et al., 2016), memory (Reul, 2014), etc. Consequently, Npas4 participates to brain responses to environmental aggression, shaping the behavioral and stress responses. Npas4 deficiency increases the vulnerability of juveniles to stress (Coutellier et al., 2015). Transgenic animals expressing various levels of Npas4 mRNA present behavioral alterations with a result depending on gene x environment interactions. Npas4$^{+/-}$ mice are prone to depression (Jaehne et al., 2015), a result congruent with the reduced Npas4 expression observed in an animal model of depression (Zhang et al., 2014), but not always found (Klaric et al., 2017). In this way, Npas4$^{-/-}$ mice exhibit high levels of spontaneous anxiety (Lin et al., 2008), an effect not observed in elevated-maze and open field testing (Coutellier et al., 2012; Jaehne et al., 2015; Klaric et al., 2017). Npas4 may also participate to the resilience after stressor exposure as it is associated to a neuroprotective activity in the brain under excessive activation such as cortical spreading depression (Hester et al., 2007), seizures (Flood et al., 2004), etc. Npas4 enhances the expression of genes (Benito and Barco, 2014) among them, those coding for brain-derived neurotrophic factor (Bdnf (Lin et al., 2008)) and activity-regulated cytoskeleton protein (Arc (Bramham et al., 2008)). Therefore, the Npas4 gene belongs to the group of "*Activity-regulated Inhibitor of Death (AID) genes*" that promote survival of the hippocampus neurons in response to synaptic NMDA stimulation (Zhang et al., 2009). This neuroprotective effect is also observed in standard living conditions. Npas4 expression is enhanced by exposure to an enriched environment (Bloodgood et al., 2013); a situation known to protect the brain of stressed animals (Cymerblit-Sabba et al., 2013; Yang et al., 2006). Conversely Npas4$^{-/-}$ mice suffer from a reduced lifespan (Lin et al., 2008; Ooe et al., 2009) and an enhanced neurodegeneration (Ooe et al., 2009).

Altogether, it suggests that Npas4 would be an important factor in stress response. Therefore, we evaluate after stressor exposure the relation between spontaneous differences in brain Npas4 mRNA expression and the stress response analyzed in terms of brain genome transcription, hormonal activation and behavior, focusing on locomotion and exploration in the elevated-plus-maze (EPM). To achieve this aim, the rats were exposed to different stressors: (i) exposure to home cage (no stressor intensity, Control, CTL), (ii) exposure to footshock apparatus (moderate stressor intensity, Sham, S), and (iii) application of footshocks (high stressor intensity, Footshock, F). We focused on the functioning of the hippocampus, a brain area involved in stress response (Pruessner et al., 2010) and frontal cortex activity regulation (Adhikari et al., 2010) with a high level of plasticity and neurogenesis (Hill et al., 2015). We evaluated the weight of Npas4 expression on the stress response by distributing stressed rats in 2 subgroups defined by their low (L) or high (H) Npas4 mRNA expression leading to 5 experimental groups (CTL, S-L, S-H, F-L and F-H). We hypothesized that animals having the highest hippocampus Npas4 mRNA expression level would be those having the highest hippocampus activation and therefore the more adjusted stress response, whatever the stressor considered.

2. Results

2.1. Clustering according to the Npas4

The S and F rats exhibited a higher expression of Npas4 mRNA than CTL rats ($F = 13.5665$, $p < .001$ with CTL *vs.* S: $p < .01$; and CTL *vs.* F: $p < .001$).

The rats were distributed into groups based on the Npas4 mRNA expression level using a clustering method without any hypothesis. The method used the Ward method for aggregation and the square Euclidian distance method for calculating distance. The 2-cluster solution was considered a compromise between precision and discrimination (Fig. 1). The animals were put into 2 subgroups according to their Npas4 mRNA level (Low Npas4 expression: L and High Npas4 expression: H). One CTL rat was not classified, due to a technical issue. Npas4 groups were further divided according to the stress exposure level. Accordingly, 6 subgroups were identified (Table 1): (i) "CTL-low Npas4" (n = 13), (ii) "CTL-high Npas4" (n = 1), (iii) "S-low Npas4" (S-L, n = 34), (iv) "S-high Npas4" (S-H, n = 6), (v) "F-low Npas4" (F-L, n = 61), (vi) "F-high Npas4" (F-H, n = 19). The "no stress" condition was considered as a unique group (CTL), independent from the Npas4 mRNA expression, because only one rat exhibited a high Npas4 value. No difference in L and H Npas4 phenotypes repartition was observed within S and F groups ($dl = 1$; $Chi^2 = 1.24$; $p = .266$), suggesting that the stressor level did not intervene in the repartition of rats within L and H subgroups.

2.2. Stress response

2.2.1. Hippocampus mRNA analyses (Fig. 2)

The factorial analysis based on all the rats isolated 4 factors: F1 (Npas4, c-fos, Arc; 33%), F2 (Hsp70-1, Bcl2; 13%), F3 (AdorA1; 10%) and F4 (Bdnf; 8%), representing 66% of the variance.

Compared to CTL rats, stressed rats exhibited an increase in brain activation observed in all 4 separate stress groups (Fig. 2, with Npas4 ($F = 67.316$, $p < .001$), c-fos ($F = 26.6856$, $p < .001$), Arc ($F = 33.347$, $p < .001$), Egr1 ($F = 7.5084$, $p < .001$), Hsp70-1 ($F = 9.0570$, $p < .001$), t-PA ($F = 8.065$, $p < 0.001$), Nf-κb1 ($F = 5.537$, $p < .001$), IκBα ($F = 9.3479$, $p < .001$), Bdnf ($F = 3.574$, $p < .01$), AdorA1 ($F = 2.8442$, $p < .05$) and Psd-95 ($F = 3.271$, $p < .05$).

Compared to the S rats, F rats exhibited higher Hsp70-1 (Stress effect: $F = 9.2844$, $p < .01$ with S-L *vs.* F-L: $p < .01$ and S-L *vs.* F-H: $p < .001$), Bdnf (Stress effect: $F = 4.825$, $p < .05$ with S-L *vs.* F-L: $p < .01$ and S-L *vs.* F-H: $p < .05$) and Psd-95 (Stress effect, $F = 5.533$, $p < .05$) mRNA expressions. The mRNA expression in the hippocampus of stressed rats also differed according to the Npas4 mRNA level. A robust Cluster effect was observed for c-fos (Cluster effect: $F = 57.4612$, $p < .001$ and Interaction: $F = 15.2551$, $p < .001$ with all *post-hoc* comparisons: $p < .01$, except S-H *vs.* F-H: $p = .050$), Arc (Cluster effect: $F = 52.730$, $p < .001$ and Interaction: $F = 8.411$, $p < .01$ with all *post-hoc* comparisons: $p < .01$; except S-H *vs.* F-H: ns), Egr1 (Cluster effect: $F = 5.0665$, $p < .05$ with S-L *vs.* F-H: $p = .054$), t-PA (Cluster effect: $F = 3.9543$, $p < .05$ with S-L *vs.* F-H: $p < .01$), Nf-κb1 (Cluster effect: $F = 21.900$, $p < .001$ and Interaction: $F = 6.780$, $p < .05$ with S-L *vs.* F-H: $p < .05$; S-L *vs.* S-H: $p < .001$ and S-H *vs.* F-L: $p < .01$), AdorA1 (Cluster: $F = 11.2568$, $p < .01$ with S-H *vs.* F-L: $p < .05$ and S-L *vs.* S-H: $p = .05$), Bcl2 (Cluster effect: $F = 3.8735$, $p = .051$ and Interaction: $F = 5.3082$, $p < .05$ with S-L *vs.* S-H: $p = .086$) and Psd-95 (Cluster effect: $F = 10.028$, $p < .01$ and Interaction: $F = 3.460$, $p = .066$ with S-L *vs.* S-H: $p < .05$ and S-H *vs.* F-L: $p < .01$). No Cluster effect and Interaction was observed for HSP70-1, IκBα and Bdnf.

Considering all the animals, the relation between Npas4 mRNA expression and the other genes was moderate for c-fos ($r^2 = 0.39$; $p < .001$) and Arc ($r^2 = 0.42$; $p < .001$) and slight for Egr1 ($r^2 = 0.20$; $p < .001$) and t-PA ($r^2 = 0.12$; $p < .001$). This masked the fact that the strength of the correlations differed according to the stress level. In CTL rats, correlations were strong for c-fos ($r^2 = 0.88$; $p < .0001$), Arc ($r^2 = 0.66$; $p < .001$) and Egr1 ($r^2 = 0.62$; $p < .001$) and slight for t-PA ($r^2 = 0.23$; $p < .0001$). In S animals, correlations were strong for c-fos ($r^2 = 0.55$; $p < .0001$) and Arc ($r^2 = 0.50$; $p < .001$), moderate for Bcl2 ($r^2 = 0.31$; $p < .001$) and slight for Nf-κb1 ($r^2 = 0.21$; $p < .01$), Psd-95 ($r^2 = 0.18$; $p < .01$)