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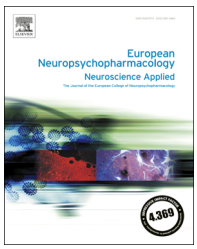

The expression of plasticity-related genes in an acute model of stress is modulated by chronic desipramine in a time-dependent manner within medial prefrontal cortex

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Abstract
It is well established that stress plays a major role in the pathogenesis of neuropsychiatric diseases. Stress-induced alteration of synaptic plasticity has been hypothesized to underlie the morphological changes observed by neuroimaging in psychiatric patients in key regions such as hippocampus and prefrontal cortex (PFC). We have recently shown that a single acute stress exposure produces significant short-term alterations of structural plasticity within medial PFC. These alterations were partially prevented by previous treatment with chronic desipramine (DMI). In the present study we evaluated the effects of acute Foot-shock (FS)-stress and pre-treatment with the traditional antidepressant DMI on the gene expression of key regulators of synaptic plasticity and structure. Expression of *Homer, Shank, Spinophilin, Densin-180*, and the small RhoGTPase related gene *Rac1* and downstream target genes, *Limk1*, *Cofilin1* and *Rock1* were investigated 1 day (1 d), 7 d and 14 d after FS-stress exposure. We found that DMI specifically increases the short-term expression of *Spinophilin*, as well as *Homer* and *Shank* family genes, and that both acute stress and DMI exert significant long-term effects on mRNA levels of genes involved in spine plasticity. These findings support the knowledge that acute FS

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stress and antidepressant treatment induce both rapid and sustained time-dependent alterations in structural components of synaptic plasticity in rodent medial PFC.

1. Introduction

Aberrant synaptic plasticity has been extensively studied in hippocampus compared to prefrontal cortex (PFC). However, recent studies have revealed that induction of synaptic plasticity mechanisms in PFC may be implicated in the pathophysiology of a number of psychiatric disorders (Goto et al., 2010). In particularly stress has been established to play a pivotal role in the pathogenesis of psychiatric disorders and to affect brain morphology by altering plasticity-related processes. Both chronic and acute stress have been shown to initiate proadaptive neuroplastic changes in the PFC, by causing shrinkage of apical dendrites and preventing small spines to mature and stabilize (Arnsten, 2009; Brown et al., 2005; Izquierdo et al., 2006; Radley et al., 2008). In this context, it is interesting that antidepressants, which have been shown to have preventive effects on stress-induced morphological alterations (Martin and Wellman, 2011), also alter the expression of plasticity-related proteins and genes, which could underlie the antidepressant effects on synaptic connectivity, as well as cognitive function and self-regulatory behaviours associated with mood disorders (Djordjevic et al., 2012; McEwen and Morrison, 2013; Sairanen et al., 2007). Scaffolding proteins located at the post-synaptic density (PSD) of glutamatergic synapses are key regulators of synaptic plasticity and dendritic spine architecture. Preclinical studies have shown that mood stabilizers including valproate and lithium modulate the expression of these structural postsynaptic genes in cortical and subcortical regions, which in turn may represent important targets for neuropsychiatric drugs (de Bartolomeis et al., 2012). Recently, we have shown that a single Foot-shock (FS) stress session affects medial PFC (mPFC) structural plasticity over time by: 1) increasing the number of small non-perforated synapses soon after stress cessation; 2) increasing spine density paralleled by decreased levels of cofilin phosphorylation and decreased apical dendritic length 24 h after stress exposure; 3) producing apical dendritic atrophy until at least 14 days after stress (Nava et al., 2015, 2014). Moreover, in the same studies the effects induced by acute FS-stress on markers of synaptic plasticity were partially prevented by prior treatment with chronic desipramine (DMI). Having observed proounced effects of acute FS-stress and DMI treatment on mPFC structural remodelling over time, including dendritic length and spine density, in the present study we aimed at investigating: the effects of acute FS-stress and pre-treatment with the traditional antidepressant DMI on the gene expression of key regulators of synaptic plasticity and structure, including *Homer* and *Shank* family genes, *Spinophilin, Densin-180,* as well as the small RhoGTPase related gene *Rac1* and downstream target genes, *Limk1*, *Cofilin1* and RhoA kinase *(Rock1)*, at distinct time points (1 day (1 d), 7 d, and 14 d after FS-stress exposure).

2. Experimental procedures

2.1. Animals and drug treatment

Male Sprague-Dawley rats (weighing 175–200 g upon arrival, from Charles River, Calco, Italy) were housed two per cage in standard propylene cages in a light controlled room (under a 12-h light/dark cycle; lights on at 7:00), at room temperature (22 °C), with food and water ad libitum.

After five days of housing, half of the animals were subjected to chronic treatment (14 days) with DMI (tricyclic antidepressant; 10 mg/kg) delivered in drinking water (vehicle). The average water intake was monitored for 5 days before and every 2 days throughout and after the treatment. Drug solutions were changed every two days according to the animals' weight and their water intake, as reported previously (Nava et al., 2015). As the animals were housed 2 per cage it was not possible to control for individual dosage. All experimental procedures involving animals were performed in accordance with the European Community Council Directive 86/609/EEC and approved by Italian legislation on animal experimentation (Decreto Ministeriale 116/1992).

2.2. Stress paradigm and corticosterone (CORT) levels

Twenty-four hours after the last drug or vehicle administration, half of the animals were subjected to a single, 40 min episode of FS-stress (20 min of total actual shock, 0.8 mA, delivered with random inter-shock length between 2–8 s) (Nava et al., 2015). After stress, animals were returned to home cages. 1 d, 7 d, and 14 d after acute FS-stress exposure, eight animals per experimental group (Vehicle/Sham; DMI/Sham; Vehicle/FS-stress; DMI/FS-stress) were rapidly euthanized by decapitation between 8:00 and 13:00 h. Trunk blood was collected shortly after decapitation, centrifuged at 1000 g at 20 °C for 20 min. Plasma was stored at −80 °C until further analysis. CORT (ng/mL) was assayed using a commercial EIA kit (Enzo Life Science). Inter- and intra-assay coefficient of variation was less than 10%.

2.3. mRNA extraction and cDNA synthesis

Shortly after decapitation and trunk blood collection, left or right hemisphere was alternatingly chosen for quantitative real-time polymerase chain reaction (real-time qPCR) analysis.

Frontal cortices were stored at −80 °C until extraction of RNA with the ParisTM RNA and protein isolation kit (Ambion, TX, USA). The isolation procedure is well-established in our laboratory and was processed as previously described (Müller et al., 2011). The integrity of RNA and the RNA concentration were determined with RNA StdSens microfluidic chips using the Experion Automated Electrophoresis System (BIORAD, CA). The RNA concentration and the purity were determined by a NanoDrop 1000 spectrophotometer (Thermo Fischer Scientific). Data on quality, concentration, and purity of the extracted RNA was evaluated with Kruskal-Wallis one-way analysis of variance (ANOVA). Before cDNA synthesis, the RNA concentration of the samples was adjusted to match the sample with the lowest concentration. RNA was reversely transcribed using