**Acute stress induces cognitive improvement in the novel object recognition task by transiently modulating *Bdnf* in the prefrontal cortex of male rats**

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**Abstract**

Stress response involves several mechanisms and mediators that allow individuals to adapt to a changing environment. The effects of stress may be adaptive or maladaptive, based on the timing and intensity of exposure as well as on the individual vulnerability. In particular, exposure to mild and brief stressors provides beneficial advantages in a short-term period, by activating protective functions to react with the external demands.

On these bases, the purpose of our study was to establish the time-dependent effects of acute stress exposure on neuroplastic mechanisms in adult male rats. Moreover, we aim at establishing the consequences of the acute challenge on memory processes by testing rats in the Novel Object Recognition (NOR) test. We found that acute restraint stress up-regulated total *Bdnf* expression 1h post stress specifically in rat prefrontal cortex, an effect that was sustained by the increase of *Bdnf* isoform IV as well as by the pool of *Bdnf* transcripts with long 3'UTR. Furthermore, in the same brain region, the acute stress modulated in a time specific manner the expression of different activity-dependent genes, namely *Arc*, *Gadd45β* and *Nr4a1*. At behavioral level, the challenge was able to improve the performance in the NOR test specifically 1h post stress, an effect that positively correlated with the expression of the neurotrophic factors.

Taken together, our results suggest that a single session of acute stress enhances memory and learning functions with a specific temporal profile, by improving neuroplastic mechanisms within the prefrontal cortex.

**Introduction**

Stress response involves several mechanisms and mediators that allow individuals to adapt to a changing environment. The effects of stress may be adaptive or maladaptive, with severe and prolonged stress underlying the development of a pathological status, whereas mild and short stress increasing the adaptive ability of the subject to cope with life stress events (McEwen 2007; McEwen et al. 2015).

In the field of psychiatric disorders, great attention has been paid in exploring the behavioral and molecular alterations driven by chronic stress exposure, with the aim to clarify the causes of their development and to assess the action of a pharmacological intervention for the identification of novel potential targets (Willner 2005; Pochwat et al. 2014; Luoni et al. 2015; Calabrese et al. 2016, 2017; Molteni et al. 2016; Rossetti et al. 2016, 2018; Yin et al. 2016). However, few attempts have been done at investigating the impact of acute stress in driving both positive and negative responses as a function of its duration and severity, thus leading to a different susceptibility and vulnerability to the challenging condition. Furthermore, stressful events have severe consequences on learning and memory and their extent has different effects at cognitive level, with impairment and improvement in the performance, respectively driven by repetitive and single stressors. Interestingly, learning under stress may facilitate memory functions (Schwabe 2017), through the involvement of several molecular pathways (Sandi and Pinelo-Nava 2007).

In this context, the neurotrophin Brain Derived Neurotrophic Factor (Bdnf), which plays a pivotal role in mechanisms of neural and synaptic plasticity (Calabrese et al. 2009; Kowiański et al. 2018), is known to be modulated by the exposure to adverse life events. Furthermore it has been shown that Bdnf is a molecular mediator of the therapeutic activity of antidepressant drugs (Duman and Monteggia 2006; Martinowich et al. 2007).

Accordingly, we showed that stress may exert opposing action on Bdnf, with chronic stress decreasing its levels (Luoni et al. 2015), whereas acute stress enhancing its expression with an anatomical specificity (Molteni et al. 2009; Fumagalli et al. 2012; Brivio et al. 2018, 2019).

Besides its role in response to stress events, Bdnf participates in long-term potentiation and it is involved in the modulation of different stages of memory processes, including acquisition, short and long term memory formation and consolidation, retrieval, extinction and reconsolidation (Bekinschtein et al. 2014; Lu et al. 2015).

On this basis, here we investigated the temporal profile of the effect exerted by 1h of restraint stress on the expression of total *Bdnf* in the rat brain. To evaluate whether the modulation of *Bdnf* was sustained by its major transcripts, we focused on the *Bdnf* long 3'UTR pool of transcripts and on *Bdnf* isoform IV that are the most abundant and well characterized transcripts in the brain areas primarily affected by stress. Bdnf long 3'UTR transcripts are targeted to distal dendrites, thereby contributing to synaptic activity as well as to long term potentiation (Lau et al. 2010; Allen et al. 2013), whereas isoform IV displays a strictly somatic localization and, as an activity-dependent gene, it is modulated by several upstream stimulatory factors (Pruunsild et al. 2011). The molecular analyses were conducted in brain areas profoundly affected by stress and regions critical also for working memory and executive functions (Stuss and Knight 2009; Kim et al. 2015), such as the prefrontal cortex (PFC) and the hippocampus (dorsal (dHip) and ventral (vHip)).

Moreover, to assess whether acute stress could alter memory functions, we investigated its impact on cognitive performance by exposing the animals to the novel object recognition test (NOR) 1, 4 and 24h after the restraint. We also established the contribution of the neurotrophin in the beneficial advantages caused by the challenge exposure in the cognitive task.

**Methods**

Animals

Adult male (post-natal day 90) Sprague-Dawley rats (Charles River, Italy) were brought into the laboratory two weeks before the start of the experiment. Rats were housed with food and water ad libitum and were maintained on a 12-h light/dark cycle and in a constant temperature (22 ± 2ºC) and humidity (50 ± 5%) conditions. All procedures used in this study are conformed to the rules and principles of the 2010/63/UE Directive, according to the authorizations from the Health Ministry. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Experimental paradigm and groups:

After two weeks, rats were randomly assigned to the following experimental groups: no stress (n=10), stress(1h)1h (n=6), stress(1h)4h (n=5), stress(1h)24h (n=5), no stress-NOR5'(n=6), no stress-NOR(n=6), stress(1h)1h-NOR5'(n=6), stress(1h)1h-NOR (n=5), stress(1h)4h-NOR (n=6), stress (1h)24h-NOR (n=7). Animals were stressed for 1h and sacrificed 1, 4 or 24h after the acute challenge, except for the animals of the no stress groups that were left undisturbed in their home cages. Moreover, half of both stressed and non-stressed animals were exposed to the novel object recognition (NOR) test 1, 4 or 24 h post-stress. During the NOR test, part of the animals was sacrificed after the first five minutes of test (NOR5'), whereas the others were killed at the end of the cognitive task (NOR).

Stress procedure

Rats were exposed to 1h of acute restraint stress, in an air-assessable cylinders (diameter 8,25 cm; length 20,32; Stereoglass Srl, Italy) (the size of the device was similar to the size of the animal, which made the animal almost immobile in the container) and sacrificed 1, 4 or 24 h after the acute challenge (fig. 1A).

Novel object recognition test

After 10 minutes of habituation in the open field in the two previous days, animals were allowed to explore two identical objects (white cylinders, 7 cm in diameter, 11 cm high) in an open field (50cm x 50cm x 40cm) for five minutes (trial session-encoding phase). After 1h in the home cage (consolidation phase), the retention trial (testing session- retrieval phase) was conducted and one of the objects presented previously was replaced by a novel object (black prism, 5 cm wide, 14 cm high) (fig. 4A). During the 5-minute test, the duration of exploration of each object (ie. sitting in close proximity to the objects, sniffing or touching them) was measured. The task was performed in an isolated room under dim light conditions, in the absence of a direct overhead lighting. The NOR discrimination index was calculated according to the following formula: time of novel object exploration minus time of familiar object exploration divided by time of novel plus familiar object exploration, multiplied by 100. The rats exposed to the cognitive test were sacrificed immediately at the end of the encoding or retention phase.

Brain tissues collection:

PFC and both dHip and vHip were dissected, frozen on dry ice and stored - 80°C for later analyses. Specifically, the PFC (defined as Cg1, Cg3 and IL subregions corresponding to the plates 6-10 according to the atlas of Paxinos and Watson (Paxinos and Watson 1986) was dissected from 2-mm-thick slices, whereas the dHip and vHip (respectively plates 25-33 and plates 34-43 according to the atlas of Paxinos and Watson) were dissected from the whole brain.

RNA preparation and gene expression analysis by quantitative Real-time PCR.

Total RNA was isolated by a single step of guanidinium isothiocyanate/phenol extraction using PureZol RNA isolation reagent (Bio-Rad Laboratories, Italy) according to the manufacturer's instructions and quantified by spectrophotometric analysis. The RNA concentrations were measured by spectrophotometry (OD260/280 1.8<ratio<2). Following total RNA extraction, the samples were processed for real-time polymerase chain reaction (RT-PCR) to assess total *Bdnf*, *Bdnf* long 3'UTR, *Bdnf* isoform IV, *Arc*, Growth Arrest and DNA Damage-inducible protein (Gadd45$\beta$ and Nuclear Receptor Subfamily 4 Group A Member 1 (*Nr4a1*). An aliquot of each sample was treated with DNase to avoid DNA contamination. RNA was analyzed by TaqMan qRT PCR instrument (CFX384 real time system, Bio-Rad Laboratories, Italy) using the iScriptTM one-step RT-PCR kit for probes (Bio-Rad Laboratories, Italy). Samples (10ng/ul) were run in 384 well formats in triplicate as multiplexed reactions with a normalizing internal control (36B4). Primers sequences (Table 1A/B) used were purchased from Eurofins MWG-Operon and Life Technologies.

Thermal cycling was initiated with an incubation at 50°C for 10 min (RNA retrotranscription) and then at 95°C for 5 min (TaqMan polymerase activation). After this initial step, 39 cycles of PCR were performed. Each PCR cycle consisted of heating the samples at 95°C for 10 s to enable the melting process and then for 30 s at 60°C for the annealing and extension reactions. A comparative cycle threshold method was used to calculate the relative target gene expression by applying the $2^{-\Delta(\Delta CT)}$ method (Livak and Schmittgen 2001).

Statistical analysis

All the analyses were conducted by using "IBM SPSS Statistics, version 24".

The behavioural data were analyzed with the one-way analysis of variance (ANOVA). When appropriate, further differences were analyzed by Fisher's Protected Least Significant Difference (PLSD). Molecular results were analyzed with the one-way (ANOVA) or two-way ANOVA, followed by PLSD. In addition, to evaluate the association between the cognitive performance and the alteration of gene expression, Pearson correlation coefficients (r) were conducted between NOR discrimination index of single animals and the corresponding mRNA levels. Significance for all tests was assumed for $p<0.05$. Data are presented as means standard error (SEM). For graphic clarity, results are presented as mean percent of No stress.

**Results**

Acute stress enhanced total *Bdnf* expression mainly in the prefrontal cortex

To investigate the time profile and the brain-region specificity of the effects mediated by a single session of a restraint stress on the expression of the neurotrophin *Bdnf*, we measured its mRNA levels at different time points in the PFC, dHip and vHip, areas known to be connected with the systems implicated in the ability to cope with external and internal challenges (Fuster et al. 2000; Kim et al. 2015).

One-way ANOVA analysis revealed a significant effect of stress on the expression of the total form of *Bdnf* specifically in the PFC ($F_{3-23}$=11.452, p=0.000) but not in the dHip and vHip (dHip: $F_{3-23}$:1.215, p=0.426; vHip: $F_{3-19}$:1.797, p=0.546) (fig.1). Post hoc analysis revealed that 1h after the stress total *Bdnf* mRNA levels significantly increased (+67%, p=0.000 vs No stress) only in PFC.

These results indicated that the acute restraint stress enhanced the expression of the total form of the neurotrophin specifically in the PFC of rats sacrificed 1h after the end of the acute stress exposure.

Acute stress exposure modulated *Bdnf* transcripts with a specific temporal profile in the prefrontal cortex.

Considering the effects of the acute stress on total *Bdnf* expression, we decided to investigate whether these changes were paralleled by a significant modulation of the major *Bdnf* transcripts, namely *Bdnf long 3'UTR* and *Bdnf isoform IV*.

In PFC, as indicated by the one-way ANOVA analysis*, Bdnf long 3'UTR* was significantly affected by stress ($F_{3-23}$:28.368, p=0.000), with an up-regulation 1h (+101%, p<0.000 vs No stress), 4h (+47%, p=0.001 vs No stress) and 24 h (+40%, p<0.002 vs No stress) post stress (fig. 2A). Similarly, acute stress significantly affected *Bdnf isoform IV* expression ($F_{3-23}$:28.393, p=0.001) (one-way ANOVA). Indeed, as shown in fig.2B, we found a significant increase in the rats sacrificed 1h (+152%, p=0.000 vs No stress), 4h (+43%, p=0.024 vs No stress) and 24h (+47%, p=0.013 vs No stress) after the end of the restraint session, with respect to non-stressed animals.

By contrast, in dHip and vHip, we found a different pattern of expression (table 2A/B). One-way ANOVA analysis showed a significant effect of stress on *Bdnf* long 3'UTR expression in both the brain regions (dHip: $F_{3-23}$:9.656, p=0.000; vHip: $F_{3-19}$:4.199, p=0.020). Accordingly, as indicated by the post hoc analysis, *Bdnf* long 3'UTR mRNA levels were significantly decreased 1h after stress (dHip:-35%, p= 0,001 vs No stress; vHip: -24%, p= 0,000 vs No stress), and of note, in dHip *Bdnf* was downregulated also 24h after the restraint (-41%, p=0,000 vs No stress).

Moreover, in vHip, Bdnf isoform IV was significantly affected by stress, as indicated by the one-way ANOVA ($F_{3-18}$:9.121, p=0.001). Indeed, as shown in table 2B, we found an up regulation of *Bdnf* IV expression 1h (+67%, p= 0.038 vs No stress), 4h (+142%, p=000 vs No stress) and 24h (+119%, p=0.001 vs No stress) post stress. Conversely, in dHip Bdnf isoform IV was not modulated by stress ($F_{3-23}$:0.171, p=0.915).

Exposure to the acute challenge up-regulated IEGs expression in a time specific manner

In order to investigate the effect of stress on neuronal activity, we focused on three activity-dependent immediate early genes *Arc*, *Gadd45β* and *Nr4a1.* In particular, *Nr4a1* adapts synaptic activity and it acts as IEG and transcription factor, responding to different stressors and stimuli (Chen et al. 2014; Helbling et al. 2014). In comparison to *Arc* and *Gadd45β* well studied genes known to be responsive to extracellular stimuli (Ma et al. 2009; Okuno 2011), *Nr4a1*, to date, has been poorly investigated as factor that displays a complex regulation under stressful situations.

As shown in fig. 3A, in PFC, *Arc* gene expression was significantly affected by stress ($F_{3\text{-}23}$:14.842, p=0.000) (one-way ANOVA). In particular, we observed an up-regulation of its mRNA levels 1h (+175%, p=0.000 vs No stress), 4h (+75%, p=0.014 vs No stress) and 24h(+88%, p=0.005 vs No stress) post stress (fig. 3A). Similarly, acute stress modulated Gadd45*β* ($F_{3\text{-}23}$:29.569, p=0.000) (fig. 3B) and *Nr4a1* ($F_{3\text{-}23}$:20.955, p<0.000) (fig. 3C) as indicated by the one-way ANOVA analysis. Indeed, the expression of both genes was up-regulated 1h (Gadd45*β*+117%, p<0.000 vs No stress; *Nr4a1:* +143%, p<0.000) and 24h (Gadd45*β:*+31%, p=0.038 vs No stress; *Nr4a1:* +74%, p=0.001) after the acute challenge.

In dHip we observed a similar pattern of activation for *Arc* and *Gadd45β,* with their mRNA levels being significantly modulated by stress ($F_{3\text{-}23}$:5.987, p=0.029; $F_{3\text{-}23}$:17.360, p=0.00 respectively) (one-way ANOVA). Indeed, we found an increased expression of Arc and Gadd45*β* 1h (+39%, p=0,010 vs No stress; +63%, p=000, respectively) and 24h (+40%, p=0,010 vs No stress; +25%, p=033, respectively) post stress (table 3A), whereas we did not find any modulation for *Nr4a1*. In vHip (table 3B), only *Arc* expression was affected by stress, as indicated by the one-way ANOVA analysis ($F_{3\text{-}19}$:7.163, p=0.002). Accordingly, its mRNA levels were increased 4h (+42%, p=0.007 vs No stress) and 24h (+61%, p=0.000 vs No stress) after the challenge. By contrast, neither *Gadd45β* nor *Nr4a1* were modulated in the vHip by 1h of restrain stress.

Acute stress enhanced the cognitive performance specifically 1h post stress

Since our molecular analysis clearly indicate that acute stress enhanced *Bdnf* expression with a specific temporal profile, and seen the well-established influence of the neurotrophin in memory and cognitive-related mechanisms (Park and Poo 2013; Kowiański et al. 2018), we investigate whether the exposure to the acute restraint stress could influence memory processes, by exposing the rats in the novel object recognition test, 1, 4 and 24 h after the challenge (fig. 4B). Exposure to 1h of acute restraint stress was associated with an improvement of the cognitive performance in the NOR test, as confirmed by the significant effect of stress ($F_{3\text{-}21}$:3.790, p=0.027). Indeed, post hoc analysis showed that animals exposed to the NOR performed significantly better when examined 1h post stress, as compared to no stress-animals tested in the NOR (+100%, p=0.018 vs No stress). By contrast, the beneficial effect of stress disappeared 4 and 24h post stress, thus underlying the transient effects of stress in promoting working memory (fig. 4C).

Correlation between *Bdnf* expression and the cognitive performance at 1h post-stress

Discrimination index was examined to investigate potential covariation within the expression levels of total *Bdnf* and *Bdnf* long 3'UTR. The analyses revealed that discrimination index positively correlated with the expression of total *Bdnf* ($r^2$ = 0.3189, p=0.004) (fig. 5A) and *Bdnf* long 3'UTR ($r^2$ = 0.1682, p=0.0465) (fig. 5B), suggesting that the enhancement of neuroplastic mechanisms may contribute to the cognitive improvement observed in acutely stressed rats.

Acute stress enhanced *Bdnf* expression during both the encoding and the retrieval phase of the NOR test.

In order to further investigate how the acute challenge affected the cognitive performance specifically 1h post-stress, when we found a major improvement of the NOR discrimination index, we assessed the expression of total *Bdnf* and *Bdnf* long 3'UTR not only at the end of the retrieval phase (NOR group), but also following the 5 minutes of encoding (NOR 5'). Two-way ANOVA analysis revealed a significant effect of stress ($F_{1-25}$:7.644, p=0.011) and test ($F_{1-25}$:20.816, p=0.000) on the total form of the neurotrophin (fig. 6A). Indeed, acute stress enhanced its expression at the end of the retrieval phase of the NOR independently from acute stress pre-exposure.

Similarly, as shown in figure 6B, *Bdnf* long 3'UTR mRNA levels were found to be affected by both stress ($F_{1-25}$:24.649, p=0.000) and test ($F_{1-25}$:26.270, p=0.001) (two-way ANOVA) .

**Discussion**

In this study, we demonstrated that acute stress modulates *Bdnf* expression with a specific temporal and anatomical profile. Moreover, at behavioral level, our results further support the concept that acute stress positively acts on cognitive performance within a precise time frame and suggests that this improvement may be related to an enhancement of neurotrophic factors.

We found that in the PFC 1h of acute stress increased the whole pool of the neurotrophin transcripts at 1h post stress, whereas 4 and 24 h later total *Bdnf* mRNA levels were not different from sham animals suggesting that, in the PFC, the acute challenge may transiently affect neuroplastic mechanisms.

Moreover, the mRNA levels of the *Bdnf long 3'UTR* pool of transcripts and of *Bdnf* isoform IV were similarly increased 1h after the stress exposure. In particular, the pool of *Bdnf* transcripts with long 3'UTR are localized to the dendritic compartment (Allen et al. 2013) and their stress-induced upregulation may contribute to the rapid transcription and translation of the neurotrophin specifically at synaptic level. Furthermore, according to the evidence that *Bdnf* isoform IV is strongly activated by depolarization of primary cortical (Martinowich et al. 2003; Pruunsild et al. 2011) and hippocampal neurons (Martinowich et al. 2003), its upregulation within 1h is in line with its role as activity dependent gene that is critical for neural plasticity.

Differently to what observed for the total form of the neurotrophin, both *Bdnf* long 3'UTR and *Bdnf* isoform IV were still upregulated 4 and 24 h following the challenge, indicating that other Bdnf isoforms may be differently modulated, thus counteracting the effect found for total *Bdnf* at these time points.

Conversely, in the dHip and vHip, total *Bdnf* was not significantly altered by stress exposure, suggesting a cortical specificity in the response to this protocol of acute challenge. Indeed, by using others paradigm it has been shown that short-time stress application increased *Bdnf* in the whole hippocampus, whereas longer stress decreased it (Marmigère et al. 2003). Interestingly, in the dHip and vHip, we found that acute stress reduced the expression of *Bdnf* long 3'UTR in both the hippocampal subregions, in line to what observed 1h post 10 minutes of forced swim stress (Shi et al. 2010) and immediately after and 24h post 1h of acute restraint stress in the CA3 and DG of the hippocampus (Murakami et al. 2005). On the contrary, only in vHip *Bdnf* isoform IV was enhanced by stress, supporting the role of this hippocampal subregion in emotion and stress, differently from the dorsal counterpart that is more related to memory and cognition (Fanselow and Dong 2010).

Furthermore, in order to investigate whether the increase of *Bdnf* was paralleled by changes of IEGs involved in activity dependent plasticity, we focused on the IEGs *Arc*, *Gadd45β* and *Nr4a1* known to be rapidly activated following acute environmental stimulations (Okuno 2011) and involved in brain functions including learning and memory (Flavell and Greenberg 2008). Similarly to what we have previously observed (Brivio et al. 2018, 2019), the strongest modulation was found in PFC, with the expression of *Arc Gadd45β* and *Nr4a1* markedly up-regulated by the acute stress. Nevertheless, the enhancement of IEGs transcription also in hippocampus is not surprising, where *Arc* and *Gadd45β* were up-regulated by stress specifically in the dorsal subregion, as already showed by Ma and colleagues (Ma et al. 2009). In particular, the peak of *Arc* mRNA levels in PFC, dHip and vHip post challenge may be due to the rapid activation of intracellular signal transduction pathways, such as the MAPK and CREB (Barry and Commins 2017). Moreover, the increased expression of *Gadd45β* and *Nr4a1* found 24h post-challenge may be mediated by several factors, such as epigenetic mechanisms (Rye et al. 2014) that operate with a different temporal profile. This effect is in line with evidence showing that the enhancement of IEGs expression is not limited to the first few hours post stress but it also occurs during resting period (Marrone et al. 2008; Barry and Commins 2017; Clayton et al. 2019).

Interestingly, since these genes are implicated in several mechanisms, including neuronal development and synaptic plasticity (Flavell and Greenberg 2008), their enhancement may contribute to different brain processes thus magnifying the consequences of the exposure to the acute challenge.

Our results support the idea that the exposure to an acute challenge leads to a complex pattern of changes that may be different depending on the brain region considered and on the function mediated by a specific intracellular mechanism in a distinct area. Additionally, these findings underlined how short stress may trigger the modulation of mechanisms of neuroplasticity mainly within the PFC, thus contributing to store information that could serve to prepare a response to a new stimulus, as for example, a cognitive task. Also, it is known that neurotrophic factors are implicated in long term potentiation and that stress may modify cognitive function through the control of Bdnf (Dragunow et al. 1993).

The role of stress in modulating learning and memory has been well described (Oitzl et al. 2001; Joëls et al. 2006), with different issues based on the type and length of stressors. Indeed, while chronic stress has detrimental effect on cognitive functions (McEwen and Sapolsky 1995), acute stress may have a dual effect: it may improve memory or, when severe, it can impair it (Hains and Arnsten 2008). In the field of adaptive response to stress, several studies have shown that stress, in close association with learning task, facilitated the memory consolidation (De Kloet et al. 1999) and might be essential for the good learning (Sandi 1997; Lupien et al. 2002). Here, we found the positive influence of 1h of acute restraint stress in modulating the cognitive performance when the animals were examined shortly after stress. This effect was not present 4h and 24h following the challenge, suggesting a transient impact of stress pre-exposure on the performance. In accordance, it has been demonstrated that 20 minutes of forced swim stress enhanced working memory in the T-maze 4 and 24 h after the stress (Yuen et al. 2009). The discrepancy between our results and the effect showed by Yuen and colleagues, found at 4 and 24 h after the challenge, may be due to the different stress used and to the different cognitive task employed. Moreover, other behavioral studies demonstrated that a single session of moderate stress facilitated classical fear conditioning (Shors et al. 1992).

At molecular level, the positive, albeit weak, correlation between NOR index and the expression of neurotrophin transcripts suggested a connection among the improvement in the cognitive performance and the specific modulation of neuroplastic mechanisms within the rat PFC, even if we are aware that further studies are needed to more directly substantiate a role for *Bdnf* in stress-related cognitive changes.

Since the NOR test consists of three steps (acquisition, consolidation, and recall) (Antunes and Biala 2012), the acute stress may impact on the behavioral outcome by acting differently on each phase of the task. Accordingly, in order to deeper investigate how stress can affect the encoding and retrieval part of the cognitive task, we focused on mechanisms of neuroplasticity exactly at the end of both the phases of NOR test.

We demonstrated that stress facilitated the cognitive performance by up-regulating the expression of the major neurotrophin transcripts during the encoding phase and additionally, *Bdnf* long 3'UTR, the pool targeted into dendrites thereby governing synaptic mechanisms (Allen et al. 2013), was further enhanced during the retrieval phase.

Taken together, the present findings suggest that acute stress was able to up-regulate the gene expression of neurotrophic factors with a precise time-profile specifically in the PFC, a brain region that is fundamental for the stress response as well as for memory encoding and retrieval (Anderson et al. 2000). Moreover, the improvement of cognitive performance due to stress exposure further highlight how stress may be a potent regulator of learning and memory, through the activation of neuroplastic factors. We believe that the characterization of the molecular mechanisms underlying the positive effect of stress exposure on memory may provide key targets to improve cognitive functions that are strongly deteriorated in neurological and psychiatric disorders.

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**Author's contributions**

FC and MAR were responsible for the study concept and design

PB and GS performed and analyzed the behavioral and the molecular analysis.

Data analysis and interpretation were done by PB and FC.

PB drafted the manuscript and FC and MAR critically revised the manuscript.

All authors critically reviewed the content and approved the final version for publication.

**Conflict of Interest**

The authors declare that they have no conflict of interest

**Statement on the welfare of animals**

All procedures used in this study involving animals have conformed to the rules and principles of the 2010/63/UE Directive, according to the authorizations from the Health Ministry n 151/2017-PR.

**Informed consent**

Informed consent was obtained from all individual participants included in the study

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**Figure Legends:**

***Fig 1:*** *Analysis of total Bdnf mRNA levels in the prefrontal cortex (PFC), dorsal (dHip) and ventral hippocampus (vHip) of acutely stressed rats, 1h, 4h and 24h after the end of the challenge (stress 1h).* Panel A represents the stress paradigm. Panel B shows total *Bdnf* expression in PFC, dHiP and vHip. The data are expressed as a percentage of No stress (set at 100%) and represent the mean ± SEM of at least 5 independent determinations. *** p<0.001 vs No stress (one-way ANOVA with Fisher's PLSD).

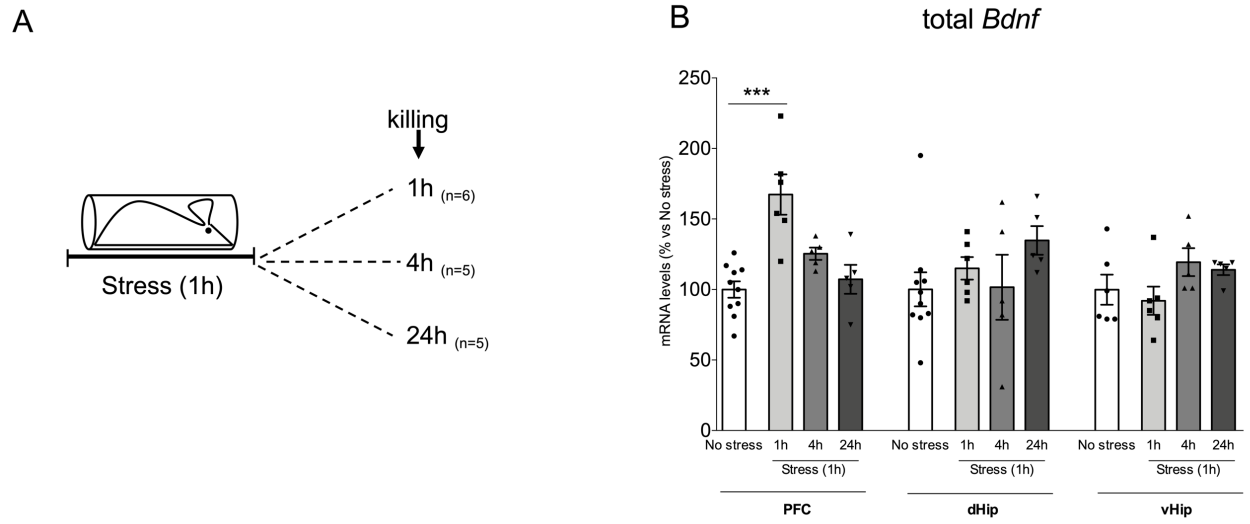


***Fig 2:*** *Analysis of Bdnf long 3'UTR (A) and Bdnf isoform IV (B) mRNA levels in the prefrontal cortex of acutely stressed rats, 1h, 4h and 24h after the end of the challenge (stress 1h).*
The data are expressed as a percentage of No stress (set at 100%) and represent the mean ± SEM of at least 5 independent determinations. * p<0.05; ** p<0.01; *** p<0.001 vs No stress (one-way ANOVA with Fisher's PLSD).

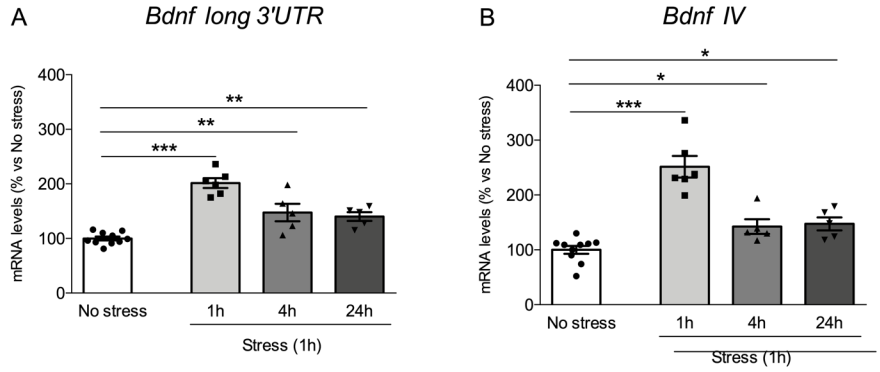


***Fig 3****: Analysis of Arc (A), Gadd45β(B) and Nr4a1 (C) mRNA levels in the prefrontal cortex of acutely stressed rats, 1h, 4h and 24h after the end of the challenge (stress 1h).*
The data are expressed as a percentage of No stress (set at 100%) and represent the mean ± SEM of at least 5 independent determinations. * p<0.05; ** p<0.01; *** p<0.001 vs No stress (one-way ANOVA with Fisher's PLSD).

**Fig.4:** *Analysis of the cognitive performance of acutely stressed animals (stress (1h)) exposed to the novel object recognition test (NOR) 1h, 4h and 24h after the end of the challenge.*
Panel A: schematic picture of the novel object recognition test; Panel B: schematic representation of the experimental paradigm; panel C: novel object recognition test (NOR) discrimination index evaluated 1h, 4h and 24h after the end of the challenge. The data, expressed as discrimination index, are the mean of at least 5 independent determinations ± SEM. * p<0.05, vs No stress (one-way ANOVA with PLSD).

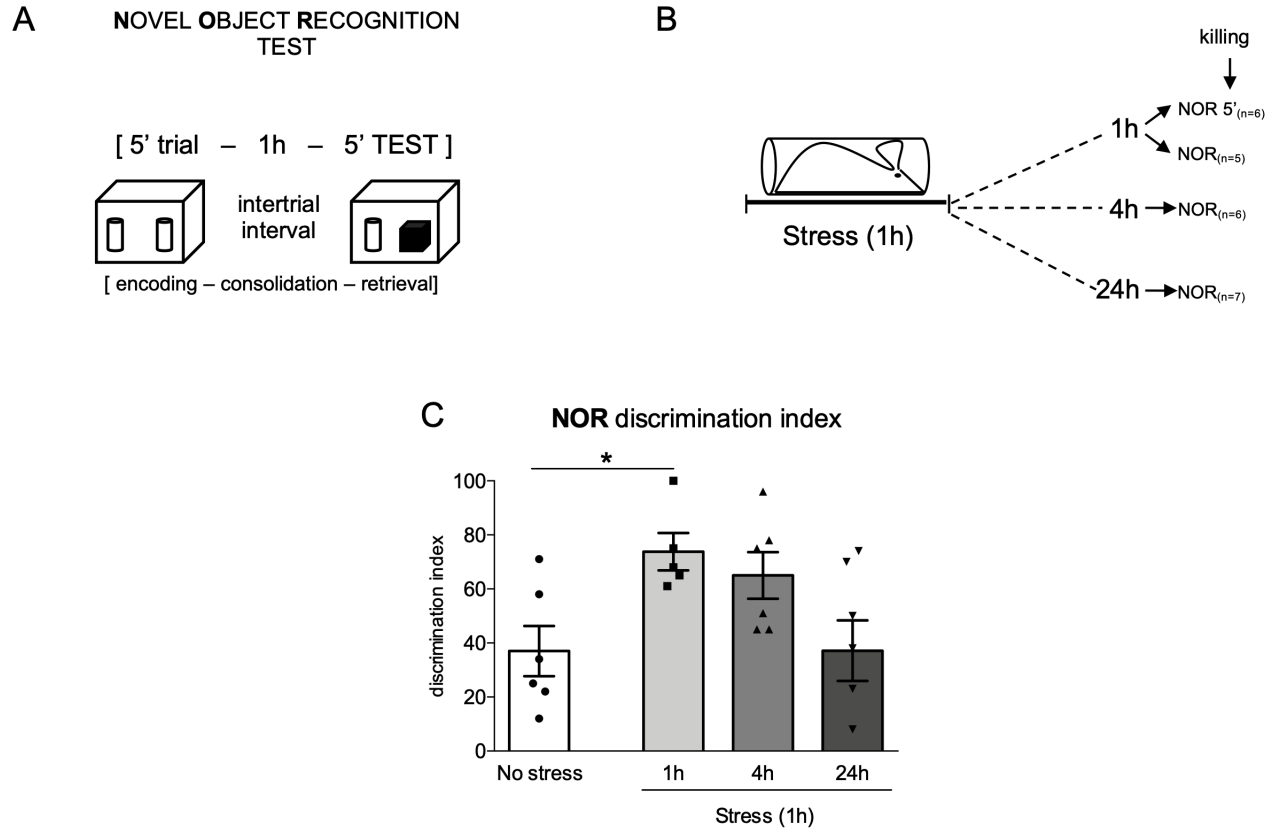


***Fig. 5:*** *Correlation between total Bdnf and Bdnf long 3'UTR mRNA levels and NOR index.* Panels A-B show the correlation analysis between the levels of total *Bdnf* (A) and *Bdnf* long 3'UTR (B) and NOR discrimination index. Analyses by Pearson's product-moment correlation ($R^2$). Data are expressed as scatterplots, with a line indicating the coefficient of the correlation between the individual data point.

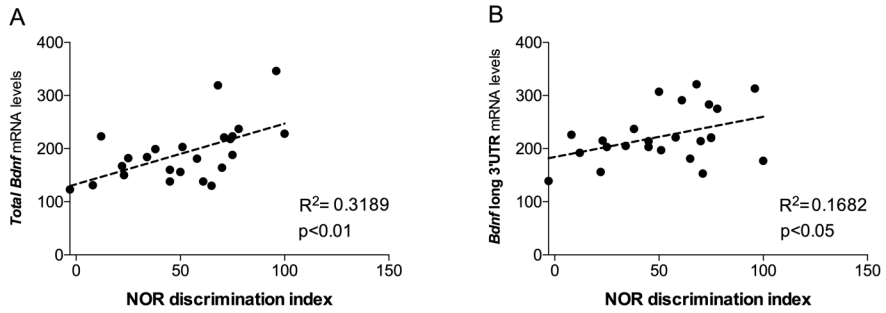

***Fig. 6:*** *Analysis of total Bdnf and Bdnf long 3'UTR in the prefrontal cortex of acutely stressed during the encoding (NOR 5') and retrieval phase (NOR) of the novel object recognition test.*
Panels A-B show the mRNA levels of *total Bdnf and Bdnf long* 3'UTR in rats exposed to 1h of acute restraint stress and tested to the novel object recognition test. The data are expressed as a percentage of No stress/NOR5' (set at 100%) and represent the mean ± SEM of at least 6 independent determinations. S: independent effect of the stress ($p<0.05$); T: independent effect of the test ($p<0.01$) (two-way ANOVA).