ORIGINAL INVESTIGATION

Basal and stress-induced modulation of activity-regulated cytoskeletal associated protein (Arc) in the rat brain following duloxetine treatment

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Abstract

Rationale Therapeutic efficacy of antidepressant drugs appears to be related to their ability in producing neuro-adaptive changes that restore normal brain function. Activity-regulated cytoskeletal associated protein (Arc) is an effector immediate early gene that plays a fundamental role in activity-dependent neural plasticity in corticolimbic brain regions and has been implicated in the modulation of several functions known to be profoundly perturbed in depressive states.

Objective In the present study, we investigated transcriptional and translational changes of Arc in response to acute or chronic treatment with the novel antidepressant duloxetine.

Results Although a limited increase of Arc messenger RNA (mRNA) levels was found in some structures after acute antidepressant administration, a marked up-regulation of its gene expression was found after chronic treatment, primarily at the level of frontal cortex. The changes observed after prolonged duloxetine administration strongly correlates with those previously reported on brain-derived neurotrophic factor mRNA levels Calabrese et al. (Neuropsychopharmacol 32:2351–2359, 2007). In addition, we found an anatomical-specific influence of chronic duloxetine on stress-dependent Arc modulation, which was limited to the frontal cortex.

Conclusions We suggest that these neuroadaptive changes, among others, might contribute to the normalization of neuroplastic defects associated with mood disorders.

Keywords Immediate early gene · Antidepressant · Frontal cortex · Synaptic plasticity · Stress

Introduction

From the discovery of the first antidepressant almost 50 years ago, a priority of research in this field has been to identify the molecular mechanisms underlying their therapeutic effects. The observation that all effective antidepressants can increase synaptic levels of serotonin and noradrenaline has led to hypothesize that major depression is due to a deficiency of monoamines (Schildkraut 1965). However, the delay between the fast synaptic effects of antidepressants and their clinical response has led to more complex hypotheses that incorporate the dysfunction at neurotransmitter levels with those on proteins that are important for cell plasticity and resiliency. In agreement with this possibility, chronic antidepressant treatment may normalize brain function through the modulation of mole-cules and systems important for neuronal plasticity (Berton and Nestler 2006; Castren 2005; Duman and Monteggia 2006). It is therefore important to improve our knowledge on molecules potentially involved in these adaptive mecha-nisms. A protein that might bridge neuronal activity with structural and functional changes is Arc or activity-regulated cytoskeletal associated protein (Lyford et al. 1995), also known as Arg3.1 (Link et al. 1995). Arc represents an interesting and important target in neuroadaptation since it is

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used to map neuronal networks that underlie information processing, and it is strongly engaged in behavioral plasticity (Tzingounis and Nicoll 2006). For example, Arc expression is regulated by long-term potentiation (LTP; Lyford et al. 1995), and in vivo infusion of Arc antisense oligonucleotides into the hippocampus interrupts the maintenance of LTP and damages long-term memory (Guzowski et al. 1999). Moreover, several physiological neural activities are able to induce Arc gene expression (Guzowski et al. 1999; Ons et al. 2004; Ramírez-Amaya et al. 2005). Since its messenger RNA (mRNA) is not only transported into dendrites, where the protein is locally synthesized, but also accumulates specifically at synaptic sites that have experienced strong activation (Steward et al. 1998), Arc levels can be considered an indicator for the coupling of synaptic activity with signaling pathways required for long-term neuronal plasticity (Bramham and Wells 2007). Although the precise function of Arc at synaptic levels is unknown, a series of recent papers have shown that Arc modulates synaptic scale of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptors by interacting with component of the endocytosis machinery to increase the rate of AMPAR endocytosis (for review, see Tzingounis and Nicoll 2006).

With regard to neuroadaptive changes involved in the action of psychotropic drugs, chronic antidepressant treatment increases Arc levels in specific regions across the rat forebrain (Pei et al. 2003), which might contribute to long-term changes on synaptic function. We aimed at expanding the possible role of Arc in neuroadaptive changes following antidepressant therapy by investigating its expression, at transcriptional and translational level, in the rat brain following single or repeated administration of the novel antidepressant duloxetine (Bymaster et al. 2005).

However, since the potential of psychotropic drugs to improve neuronal plasticity might also reside in the ability to modulate a specific system under a challenging condition, we investigated the potential of antidepressant treatment to modulate rapid Arc changes following exposure to stress, which represents a major precipitating element in psychiatric disorders.

Materials and methods

Materials

General reagents were purchased from Sigma-Aldrich (Milan, Italy), and molecular biology reagents were obtained from Ambion (Austin, TX, USA), New England Biolabs (Beverly, MA, USA), Promega (Milan, Italy), and Bioline (London, UK). Duloxetine was obtained from Eli Lilly & Co. (Indianapolis, IN, USA).

Animals

Male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 225–250 g were used throughout the experiments. Rats were housed for 2 weeks before any treatment and maintained under a 12-h light/12-h dark cycle with food and water available ad libitum.

The first analysis was focused on the modulation of Arc by duloxetine, whereas in the second part of this study, acute stress was used as 'challenge' to investigate the influence of chronic duloxetine on the stress-dependent modulation of Arc.

Pharmacological treatments and stress paradigm

To evaluate the effect of duloxetine on Arc expression, the following experimental groups were used: vehicle, animals treated for 21 days with vehicle solution; acute duloxetine, animals received the vehicle for 20 days and a single administration of duloxetine (10 mg/kg), on the 21st day, and were killed 1 h later; and chronic duloxetine, animals received daily administration of duloxetine (10 mg/kg) for 3 weeks and were killed 1 or 24 h after the last administration of the drug. Vehicle and duloxetine were administered by gavage.

For the stress experiment, animals chronically treated with vehicle or duloxetine were exposed 24 h after the last drug injection to an acute stress consisting of a 5-min forced-swim stress. One half of each group was left undisturbed in their cages (No stress), whereas the second half was placed 24 h after the last drug administration in plexiglass cylinders filled with 30 cm water at 25°C. Animals were sacrificed 15 min after the end of the stress session.

Animals were killed by decapitation, and the brain regions of interest were rapidly dissected. Entorhinal cortex, hippocampus, striatum, midbrain, and hypothalamus were dissected from the whole brain, whereas prefrontal cortex (defined as Cg1, Cg3, and IL subregions corresponding to the plates 6–10 according to the atlas of Paxinos and Watson), frontal cortex (comprising regions Fr1–3, from the same slice as prefrontal cortex), and parietal cortex (Par1–Par2 from plates 10 to 17) were dissected from 2-mm-thick slices (Paxinos and Watson 1996). The brain specimens were frozen on dry ice and stored at −80°C for further analysis. All animals handling and experimental procedures were performed in accordance with the EC (EEC Council Directive 86/609 1987) and the Italian legislation on animal experimentation (Decreto Legislativo 116/92).

RNA preparation

Tissue from different brain structures was homogenized in 4 M guanidinium isothiocyanate (containing 25 mM sodium

citrate pH 7.5, 0.5% sarcosyl, and 0.1% 2-mercaptoethanol). Total RNA was isolated by phenol/chloroform extraction, and quantification was carried out by spectrophotometric analysis.

RNase protection assay

Arc gene expression analysis was performed on total RNA by RNase protection assay. A transcription kit (MAXI script; Ambion) was used to generate complementary RNA (cRNA) probes from Arc cDNA plasmid (a generous gift of Dr. P. Worley, Johns Hopkins University, Baltimore, MD, USA) and β-actin-Rat (Ambion), and ^{32}P-CTP was utilized as a radiolabeled nucleotide. The cRNA probes and the relative protected fragment (pf) were as follows: Arc= 630 bp, pf=620 bp; β-actin=164 bp, pf=126 bp.

The RNase protection assay was carried out on a 10-μg sample of total RNA as described previously (Riva et al. 1996). Briefly, after ethanol precipitation, total RNA was dissolved in 20 μl of hybridization solution [80% formamide, 40 mM piperazine-1,4-bis(2-ethanesulfonic acid) pH 6.4, 400 mM sodium acetate pH 6.4, and 1 mM EDTA] containing 50,000 cpm of ^{32}P-labeled Arc cRNA probe (specific activity >10^8 cpm/μg) and 50,000 cpm of ^{32}P-labeled β-actin probe. After being heated at 85°C for 10 min, the cRNA probes were allowed to hybridize to the endogenous RNAs at 45°C overnight.

At the end of hybridization, the solution was diluted with 200 μl of RNase digestion buffer (300 mM NaCl, 10 mM Tris HCl, pH 7.4, and 5 mM EDTA, pH 7.4) containing a 1:400 dilution of a RNase cocktail (1 mg/ml RNase A and 20 U/ml RNase T1) and incubated for 30 min at 30°C. Proteinase K (10 μg) and sodium dodecyl sulfate (SDS; 10 μl of 20% stock solution) were then added to the sample, and the mixture was incubated at 37°C for an additional 15 min. At the end of incubation, the sample was extracted with phenol/chloroform and ethanol-precipitated. The pellet, containing the RNA/RNA hybrids, was dried and resuspended in loading buffer (80% formamide, 0.1% xylene cyanol, 0.1% bromophenol blue, and 2 mM EDTA), boiled at 95°C for 5 min, and separated on 5% polyacrylamide gel under denaturing conditions (7 M urea). The protected fragments were visualized by autoradiography.

Preparation of protein extract and western blot analysis

Tissues were homogenized in a glass–glass potter in cold 0.32 M sucrose solution containing 1 mM HEPES, 0.1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, pH 7.4, in the presence of a complete set of protease inhibitors and a phosphatase inhibitor cocktail. The homogenized tissue was centrifuged at 1,000×g for 10 min to obtain a pellet (P1), corresponding to the nuclear fraction; the supernatant (S1)

was centrifuged at 9,000×g for 15 min to obtain a clarified fraction of cytosolic proteins (S2) and the pellet (P2), corresponding to the crude synaptosomal membrane where, after dissolution in a buffer (20 mM HEPES, 0.1 mM DTT, 0.1 mM EGTA) with protease and phosphatase inhibitors, the levels of Arc were analyzed by western blot.

Total protein content was measured according to the Bradford Protein Assay procedure (Bio-Rad, Milan, Italy) using bovine serum albumin as calibration standard. After adjusting total protein concentrations to the same amount (10 μg), all samples were run on a SDS–10% polyacrilamide gel under reducing conditions, and proteins were then electrophoretically transferred onto nitrocellulose membranes (Amersham). Blots were blocked with 10% nonfat dry milk and then incubated with primary anti-Arc polyclonal antibody (1:4,000, 1 h at room temperature; a generous gift of Dr. P. Worley, Johns Hopkins University). Membranes were then incubated for 1 h at room temperature with a 1:2,000 dilution of peroxidase-conjugated anti-rabbit IgG (Cell Signaling), and Arc immunocomplexes were visualized by chemiluminescence utilizing the ECL Western Blotting kit (Amersham Life science, Milan, Italy) according to the manufacturer's instructions. Arc protein was detected by evaluating the band density at 55 kDa.

Results were standardized to β-actin, as control protein, which was detected by evaluating the band density at 43 kDa after probing the membranes with a polyclonal antibody (1:10,000 dilution, Sigma) followed by a 1:10,000 dilution of peroxidase-conjugated anti-mouse IgG (Sigma).

Correlation analysis

Based on the close interaction between Arc and the neurotrophin brain-derived neurotrophic factor (BDNF; Bramham and Messaoudi 2005), we examined the possible relationship between the changes induced by duloxetine on Arc (present manuscript) and BDNF (Calabrese et al. 2007) mRNA levels measured in the same rat by RNase protection assay. Since both acute and chronic duloxetine modulated Arc gene expression in frontal cortex, entorhinal cortex, and midbrain, the corresponding percent changes were plotted against the matching BDNF values (published results in Calabrese et al. 2007; unpublished results for midbrain), and the Pearson product moment correlations (r) were calculated.

Densitometric and statistical analyses

The levels of mRNA and protein for Arc and β-actin were calculated by measuring the optical density of the autoradiographic bands using Quantity One software (Bio-Rad). To ensure that autoradiographic bands were in the linear range of intensity, different exposure times were used. β-Actin

was employed as internal standards for the RNase protection assay and western blot since its expression was not regulated by acute or chronic duloxetine treatment. The effects of acute and chronic treatments with duloxetine were analyzed with one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference. Conversely, the data obtained from the stress experiment were analyzed by two-way ANOVA, with drug (vehicle vs. duloxetine) and treatment (no stress vs. stress) as independent factors and Arc levels as dependent variable. When appropriate, further differences were analyzed by single-contrast post hoc test (SCPHT). Data are presented as means±standard error (SEM), with each individual group comprising at least six independent samples. For graphic clarity, optical densities from experimental groups were expressed and presented as mean percent of control group, namely the group that received only the vehicle in pharmacological experiments and the non-stressed vehicle-treated rats in the stress experiments. Significance for all tests was assumed at least at $p<0.05$.

Results

In the present study, we investigated the expression of the effector immediate early gene Arc in rats treated with the novel antidepressant duloxetine in order to identify possible differences in its anatomical distribution and subcellular localization. In addition, we investigated the influence of duloxetine on Arc expression following an acute stress.

A single injection of duloxetine regulates Arc gene expression in a region-specific manner (Fig. 1). One hour after drug administration, we observed a marked and significant reduction of Arc mRNA levels in frontal cortex (-45%, $p=0.013$), whereas its expression was strongly up-regulated in entorhinal cortex ($+64\%$, $p=0.021$) and, to less extent, in the midbrain ($+41\%$, $p=0.003$), which comprises major dopaminergic cell bodies. No significant changes were instead found in prefrontal and parietal cortex, in hippocampus, a region with a major role in mood disorders and antidepressant response, as well as in striatum and hypothalamus.

A different expression profile of Arc mRNA was observed after prolonged duloxetine treatment (21 days), depending on whether the rats were killed 1 or 24 h after the last drug administration. As summarized in Fig. 2, an overall increase of Arc mRNA levels was found in the brain of chronically treated animals killed at 1 h, whereas its expression was mainly down-regulated 24 h later. In more detail, rats killed 1 h after the last drug injection displayed major changes at cortical level: Arc was strongly up-regulated in frontal cortex ($+146\%$, $p<0.001$) and significantly increased also in entorhinal cortex ($+69\%$, $p=0.014$) and in prefrontal cortex ($+19\%$, $p=0.045$). With the exception of the midbrain, where we found a significant increase of Arc ($+33\%$, $p=0.004$), we did not observe any significant change in the other brain regions examined. As already mentioned, a different situation was observed 24 h later. The mRNA levels for the activity-regulated gene were back to control levels in most structures, including frontal cortex, entorhinal cortex, prefrontal cortex, and midbrain, or even significantly reduced in others (parietal cortex, -37%, $p=0.003$; striatum, -26%, $p=0.04$).
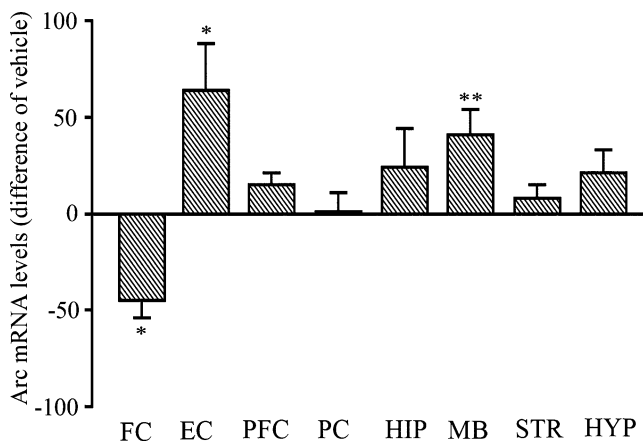

Fig. 1 Analysis of Arc mRNA levels in different rat brain structures 1 h after a single injection of duloxetine. The results, expressed as percentage difference of vehicle-injected rats, represent the means± SEM of at least seven independent determinations in the following regions: *FC* frontal cortex, *EC* entorhinal cortex, *PFC* prefrontal cortex, *PC* parietal cortex, *HIP* hippocampus, *MB* midbrain, *STR* striatum, *HYP* hypothalamus. *$p<0.05$ and **$p<0.001$ vs. vehicle-injected rats (one-way ANOVA)
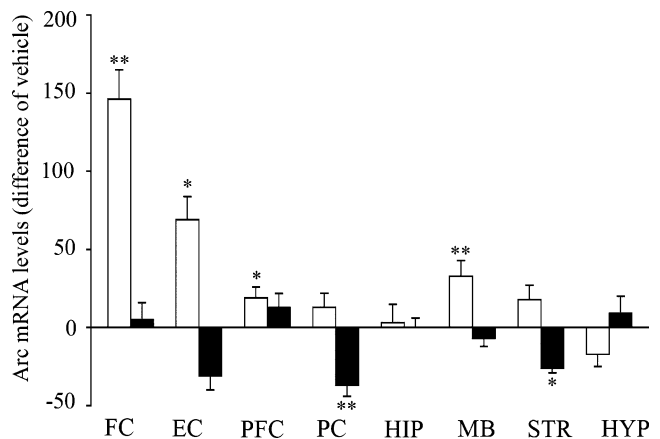

Fig. 2 Anatomical profile of the effects produced by chronic treatment (21 days) with duloxetine on Arc gene expression. Animals were killed 1 (*open bars*) or 24 h (*closed bars*) after the last drug administration. Data, expressed as percent difference of vehicle-injected rats, represent the means±SEM of at least eight independent determinations in the following regions: *FC* frontal cortex, *EC* entorhinal cortex, *PFC* prefrontal cortex, *PC* parietal cortex, *HIP* hippocampus, *MB* midbrain, *STR* striatum, *HYP* hypothalamus. *$p<0.05$ and **$p<0.001$ vs. vehicle-received rats (one-way ANOVA)

We recently demonstrated that chronic duloxetine treatment modulates BDNF mRNA expression in a region-specific manner (Calabrese et al. 2007). Based on the close interaction between BDNF and Arc (Bramham and Messaoudi 2005), we examined the possible covariation in the expression of these two genes in cortical regions and midbrain. The analyses revealed that no correlation between Arc and BDNF mRNA levels was found after a single administration of the antidepressant ($r=0.296$, $p=0.106$; Fig. 3a), suggesting that the changes of Arc expression observed after one injection are most likely unrelated to BDNF. Conversely, after chronic duloxetine treatment (Fig. 3b), a strong positive correlation was found between Arc and BDNF mRNA levels in the different brain regions ($r=0.913$, $p<0.01$).

Since the potential of antidepressant drugs to improve neuronal plasticity might also be related to the modulation of specific neuronal systems under a challenging condition, we measured Arc expression in rat chronically treated with duloxetine and exposed to an acute swim stress. When comparing the modulation of Arc mRNA levels in control vs. duloxetine-treated rats, we found that the up-regulation of its expression by stress was not affected by chronic antidepressant treatment. In fact a significant effect of stress was detected in frontal cortex ($F_{1,49}=33.622$, $p=0.0001$), prefrontal cortex ($F_{1,44}=75.154$, $p<0.0001$), and hippocampus ($F_{1,41}=71.160$, $p<0.0001$), but no significant treatment×stress interaction was observed in any of these structures. Accordingly, as shown in Fig. 4, a significant increase of Arc mRNA levels was found following forced-swim stress in frontal cortex (vehicle: +92%, $p=0.0001$; duloxetine: +86%, $p=0.002$, two-way ANOVA with SCPHT; Fig. 4b), prefrontal cortex (vehicle: +144%, $p<0.0001$; duloxetine: +108%, $p<0.0001$, two-way ANOVA with SCPHT; Fig. 4c), and hippocampus (vehicle: +65%, $p<0.0001$; duloxetine: +54%, $p<0.0001$, two-way ANOVA
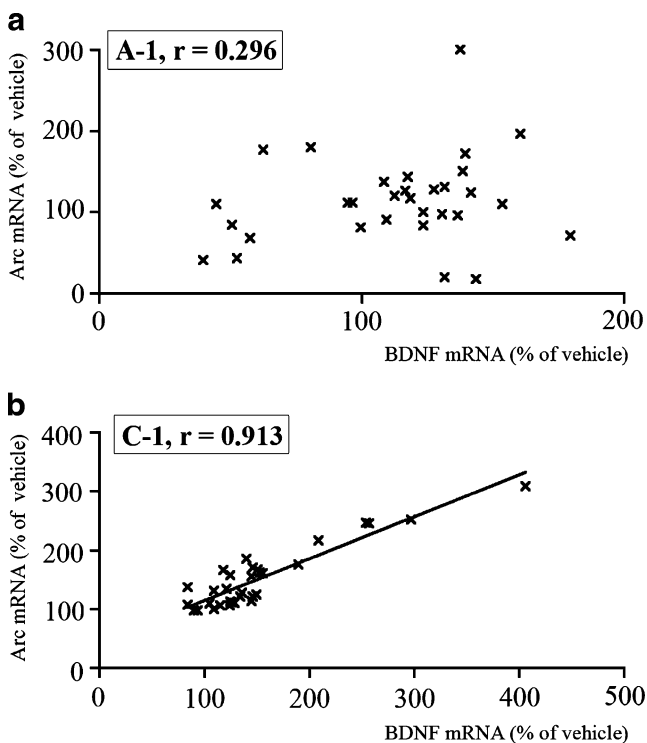

Fig. 3 Correlation analysis between Arc and BDNF mRNA changes in cortical region regions (frontal cortex and enthorinal cortex) and midbrain after a single injection (**a**) or a chronic treatment (**b**) with duloxetine. Data points in plots indicate the amount of Arc (present manuscript) and BDNF (results for frontal and enthorinal cortex published in Calabrese et al. 2007; unpublished results for midbrain) mRNA levels per rat, expressed as percentage of control animals. Analyses by Pearson product moment correlation (r)
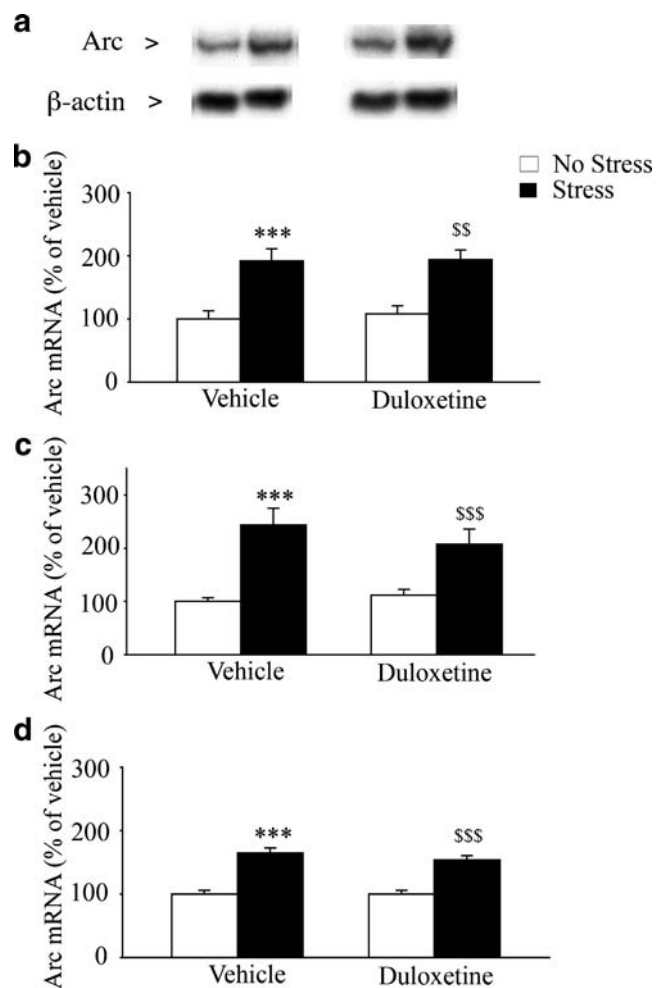

Fig. 4 Effect of acute swim stress on Arc gene expression measured in rats chronically treated with duloxetine (10 mg/kg) or vehicle and killed 15 min after the end of the stress session. **a** Representative bands from RNase protection assay performed in frontal cortex. Quantitative data are illustrated for frontal cortex (**b**), prefrontal cortex (**c**), and hippocampus (**d**). The data represent Arc mRNA levels expressed as percentage of controls (un-stressed animals treated with vehicle, set at 100%). *Bar graphs* are the mean±SEM from eight independent determinations. ***$p<0.0001$ vs. vehicle- and $^{\$\$}p<0.001$, $^{\$}p<0.0001$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT)

with SCPHT; Fig. 4d) of both experimental groups (vehicle- and duloxetine-treated rats).

On the contrary, we found that duloxetine treatment regulated stress-dependent changes of Arc protein in frontal cortex, as demonstrated by a significant duloxetine × stress interaction ($F_{1,18}=7.150$, $p=0.018$; Fig. 5b). In this brain region, the acute swim stress reduced Arc protein levels in vehicle pre-treated rats (−25%, $p=0.046$), but not in animals that were pre-treated with duloxetine. In prefrontal cortex (Fig. 5c), Arc was up-regulated by duloxetine independently from stress exposure ($F_{1,20}=9.498$, $p=0.007$ main effect of duloxetine), whereas the acute stress

per se did not alter protein levels of the early inducible gene. Finally, no changes were found in all the experimental groups at hippocampal level (Fig. 5d).

Discussion

Our results demonstrate that duloxetine, a novel antidepressant acting as serotonin and noradrenaline reuptake inhibitor, significantly affects the expression of Arc both at transcriptional and translational level in selected rat brain regions.

Although cloned almost 10 years ago, the functional role of Arc in the brain is still poorly understood. Because this effector immediate early gene is rapidly activated by synaptic activity (Guzowski 2002; Tzingounis and Nicoll 2006), its expression has been extensively used as a molecular marker linking the activity of neural circuitry to behavior (Guzowski 2002; Temple et al. 2003; Tagawa et al. 2005).

The analysis of Arc expression after acute and chronic manipulations (antidepressant treatment in the present study) may provide information regarding the extent of re-modeling and responsiveness ongoing in specific brain structures. On this basis, the up-regulation of Arc in entorhinal cortex and midbrain, which is similar after single or repeated antidepressant treatment, might reflect direct synaptic changes produced by duloxetine (blockade of serotonin and noradrenaline transporters). Conversely, the marked increase observed in the frontal cortex of chronically treated rats most likely represents a neuroadaptive event that might be part of the cytoarchitectural re-modeling occurring in response to repeated administration of antidepressant drugs (Pittenger and Duman 2008). Twenty-four hours after the end of chronic duloxetine treatment, Arc mRNA expression leveled off or was decreased below controls in the brain regions where the gene was up-regulated at 1 h. Although chronic electro-convulsive therapy reduces Arc mRNA levels at 24 h (Larsen et al. 2005), differences exist with respect to other antidepressants that up-regulate its expression several hours after the end of chronic administration (Pei et al. 2003). Such discrepancies might be explained by differences in the experimental procedures, primarily length of treatment and time of sacrifice, but it does not appear to be related to the antidepressant used since serotonin and noradrenaline reuptake blockers produce similar changes on Arc expression (Pei et al. 2003). We found a strong correlation between BDNF and Arc expression in cortical regions after chronic, but not acute, duloxetine treatment. Since the neurotrophin can increase the expression of Arc (Ying et al. 2002; Rao et al. 2006), we suggest that the alterations of Arc expression might contribute to neuronal re-modeling
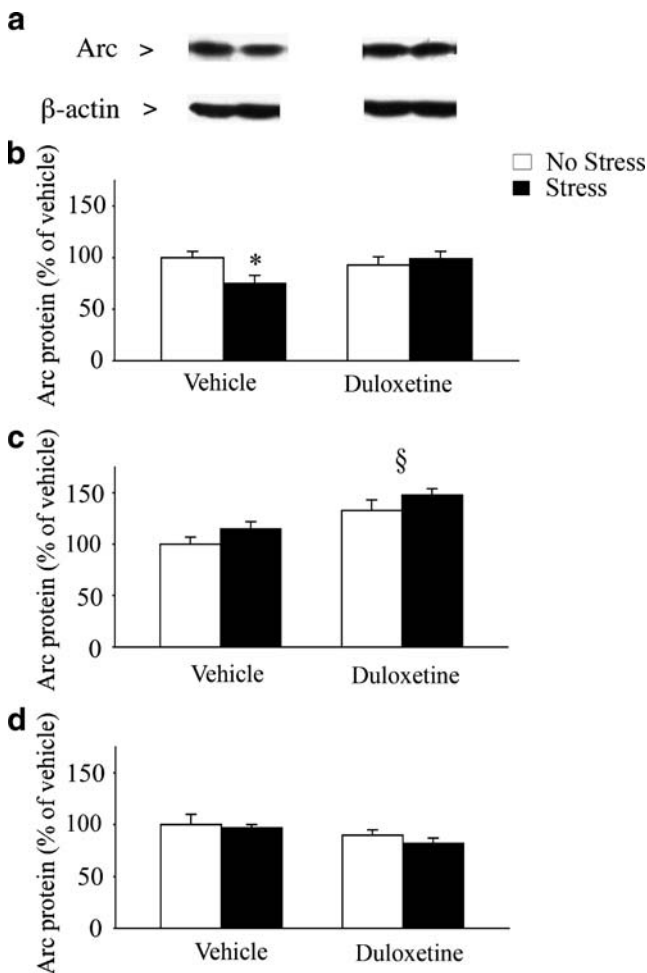

Fig. 5 Effect of acute swim stress on Arc protein levels measured in rats chronically treated with duloxetine (10 mg/kg) or vehicle and killed 15 min after the end of the stress session. a Representative bands from western blotting performed in frontal cortex. Quantitative data are illustrated for frontal cortex (b), prefrontal cortex (c), and hippocampus (d). The data represent Arc protein levels expressed as percentage of controls (un-stressed animals treated with vehicle, set at 100%). Bar graphs are the mean±SEM of four to six independent determinations. *$p<0.05$ vs. vehicle-treated rats (two-way ANOVA with SCPHT) and §$p<0.05$ vs. vehicle-treated rats (two-way ANOVA, main effect of duloxetine)

triggered by the neurotrophin (Bramham and Messaoudi 2005, Bramham and Wells 2007) and can be important for the antidepressant response at cortical level. As such correlation was not observed in chronically treated animals killed 24 after the last injection (data not shown), we hypothesize that the effect observed at 1 h may represent a coincident mechanism observed in response to antidepressant injection after chronic priming of the system.

Since one major element of vulnerability in mood disorders is stress, we further investigated whether chronic antidepressant treatment might affect the modulation of Arc in response to an acute stress. In line with previous evidence (Ons et al. 2004), even our mild and short-lasting stress produces a rapid increase of Arc mRNA levels in frontal and prefrontal cortex as well as in the hippocampus. This effect is qualitatively and quantitatively similar in duloxetine-treated rats, suggesting that chronic antidepressant exposure does not alter the rapid transcription of the IEG in response to a challenging situation. Since time-dependent changes in Arc modulation have been reported (Ramírez-Amaya et al. 2005; Trnecková et al. 2007), we cannot rule out the possibility that a distinct temporal profile of Arc regulation by stress is found after antidepressant treatment.

Conversely, duloxetine treatment prevented the stress-induced reduction of Arc protein in the synaptic compartment, an effect restricted to frontal cortex. This suggests that rapid 'synaptic' changes of Arc protein may occur in response to stress and that these changes can be prevented by chronic antidepressant treatment. We can envisage that this effect might reflect a specific difference in the expression or function of systems involved in Arc translation at synaptic level. Alternatively, although the Arc half-life is not known, we cannot exclude that other mechanisms, including protein degradation or redistribution, may contribute, at least in part, to the stress-induced reduction of Arc at synaptic level.

While small changes in the levels of Arc mRNA levels were found in the prefrontal cortex of duloxetine-treated rats, we observed a significant increase of its protein in the crude synaptosomal fraction. The increased levels of Arc protein in this compartment can be sustained by local translation of its mRNA, possibly through the action of neurotransmitter and hormonal systems (Steward and Worley 2001) as well as neurotrophic pathways (Calabrese et al. 2007; Bramham and Wells 2007) known to regulate Arc. A sustained translation of the activity-regulated gene can participate in neuroplastic mechanisms involved in neuronal consolidation (Tzingounis and Nicoll 2006) but might also regulate the trafficking of AMPA glutamate receptors at synaptic level (Shepherd et al. 2006).

It is well known that several functions require accurate temporal processing of specific input in order to orchestrate the appropriate output. Based on that, our data suggest that the transcriptional and translational effects produced by duloxetine on the expression of the effector immediate early gene Arc, primarily after chronic treatment, may reflect structural and functional changes supporting an increased neuronal plasticity that might contribute to the amelioration of functions that are defective in depressed subjects.

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