Progress in Neuro-Psychopharmacology & Biological Psychiatry 34 (2010) 122–130

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Chronic psychosocial stress alters NPY system: Different effects in rat and tree shrew

E. Zambello [a,b,*], E. Fuchs [c,d], N. Abumaria [c,d,e], R. Rygula [c,d], E. Domenici [a], L. Caberlotto [a]

[a] Neurosciences Centre for Excellence in Drug Discovery, Mood & Anxiety Disorders DPU, GlaxoSmithKline Medicines Research Center, Verona, Italy
[b] Section of Pharmacology, Department of Medicine and Public Health, University of Verona, Italy
[c] Clinical Neurobiology Laboratory, German Primate Center, Göttingen, Germany
[d] DFG Research Center Molecular Physiology of the Brain, University Göttingen, Göttingen, Germany
[e] Center for Learning and Memory, School of Medicine B.303, Tsinghua University, 100084 Beijing, China

ARTICLE INFO

Article history:
Received 4 June 2009
Received in revised form 13 October 2009
Accepted 14 October 2009
Available online 19 October 2009

Keywords:
Animal model
Antidepressant
Depression
Fluoxetine
In situ hybridization
Neuropeptide

ABSTRACT

The neuropeptide Y (NPY) system has been largely studied in relation to affective disorders, in particular for its role in the mechanisms regulating the pathophysiology of anxiety and depression and in the stress-related behaviours. Although NPY has been previously investigated in a variety of animal models of mood disorders, the receptor subtype mainly involved in the modulation of the stress response has not been identified. In the present study, the chronic psychosocial stress based on the resident–intruder protocol—an ethologically relevant paradigm known to induce behavioural and endocrine modifications which mimic depression-like symptoms—was used. Two different species were investigated: rat and tree shrew (*Tupaia belangeri*); the latter is regarded as an intermediate between insectivores and primates and it was chosen in this study for its pronounced territoriality. In these animals, the regulation of NPY and of Y_1, Y_2 and Y_5 receptors mRNA expression was evaluated after chronic stress and chronic antidepressant treatment by *in situ* hybridization in selected brain regions known to be involved in the pathophysiology of mood disorders. The animals were exposed to psychosocial stress for 35 days and concomitant daily fluoxetine treatment (10 mg/kg for rats and 15 mg/kg for tree shrews) after the first week of stress. The results confirmed a major role for hippocampal and hypothalamic NPY system in the pathophysiology of mood disorders. Although there were no evident differences between rat and tree shrew in the NPY system distribution, an opposite effect of chronic psychosocial stress was observed in the two species. Moreover, chronic antidepressant treatment was able to counteract the effects of stress and restored basal expression levels, suggesting the utility of these paradigms as preclinical models of stress-induced depression. Overall, although evident species differences were found in response to chronic psychosocial stress, the present study suggests a role for NPY receptors in the stress response and in the action of antidepressant drugs, providing further support for an involvement of this neuropeptidergic system in the pathophysiology of depression and anxiety.

1. Introduction

The study of the mechanisms involved in the etiology of affective disorders requires the investigation of animal models potentially reflecting the course and symptoms of human pathologies. In addition to genetic factors, which are known to predispose to psychopathologies (McGuffin and Katz, 1989), environmental stress plays an important role in the etiology of anxiety and depression, which are linked to maladaptive changes in the stress response (Kendler et al., 1995a,b). Since the majority of stressful stimuli leading to psychopathologies in humans are of social nature (Bjorkqvist, 2001; Kendler et al., 2003; Kessler, 1997; Kessler et al., 1985), the study of the consequences of social stress in experimental animal models is of great interest. Social defeat using the resident–intruder paradigm (Tornatzky and Miczek, 1994) represents a suitable and naturalistic experimental method to study the effects of stress (Fuchs et al., 1996). Subordinate animals exhibit physiological and behavioural changes similar to those of depressive-like state, such as increased adrenocorticotropin hormone (ACTH) and glucocorticoid activity (Buwalda et al., 1999, 2001), disturbances in sleep (Rüther, 1989), altered heart rate, blood pressure and core temperature (Meerlo et al., 1996; Sgoifo et al., 1999), impaired immunological function and reduced resistance to diseases (Engler et al., 2004; Stefanski and Engler, 1998), decreased locomotor and exploratory activities (Koolhaas et al., 1997; Meerlo et al., 1996; Rygula et al., 2005), reduced self-grooming (van Erp et al., 1994), impaired consummatory behaviour, with a consequent loss of body weight (Kramer et al., 1999; Rybkin et al., 1997), reduced aggression and sexual behaviour

Abbreviations: ACTH, adrenocorticotropin hormone; ANOVA, analysis of variance; CA1, CA1 region of hippocampus; CA2, CA2 region of hippocampus; CA3, CA3 region of hippocampus; CNS, central nervous system; DG, dentate gyrus; HPA, hypothalamic-pituitary-adrenocortical; NPY, Neuropeptide Y; PBS, phosphate buffered saline; SSC, saline sodium citrate; VMH, ventro-medial hypothalamus; VMHDM, ventro-medial hypothalamus, dorso-medial portion.

* Corresponding author. Neurosciences Centre for Excellence in Drug Discovery, Mood & Anxiety Disorders DPU, GlaxoSmithKline Medicines Research Center, Verona, Italy.
E-mail address: erika.2.zambello@gsk.com (E. Zambello).

0278-5846/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2009.10.011

(McGrady, 1984), increased submissive behaviour and anxiety (Ruis et al., 1999).

Neuropeptide Y (NPY) is a neuroactive peptide acting as a neurotransmitter and neuromodulator regulating many physiological functions (Colmers and Wahlestedt, 1993). Preclinical and clinical studies support the role of NPY in the regulation of emotions and stress-related behaviours, showing that increased NPY levels in specific regions of the central nervous system (CNS) induce antidepressant- and anxiolytic-like effects, whereas a down-regulation of this peptide induces opposite effects on the emotional responses (Heilig, 2004). Since the NPY system is altered by stressful challenges (Castagné et al., 1987), the study of its expression in animal models of depression could be useful in clarifying the role of NPY in emotional behaviours. Previous studies on genetic models (Bjørnebekk et al., 2006; Caberlotto et al., 1998, 1999; Husum et al., 2008, 2001; Jiménez-Vasquez et al., 2000a,b; Wortwein et al., 2006) or environmental models (Husum and Mathé, 2002; Husum et al., 2002; Jiménez-Vasquez et al., 2001) have demonstrated alterations of the NPY system in the CNS; however, the variety of regions affected by changes does not allow a simple interpretation, although the hippocampus seemed to be consistently involved.

Of the seven NPY receptor subtypes isolated to date, named Y_1–Y_7, a number of findings have indicated Y_1 and Y_2 receptors as the main modulators of NPY anti-stress activity (Heilig, 2004). The Y_1 receptor agonists exert an antidepressant- and anxiolytic-like action (Ishida et al., 2007), while the Y_1 antagonists induce anxiety and depressive-like behaviour in various animal models of anxiety (Primeaux et al., 2005; Redrobe et al., 2002; Sajdyk et al., 1999). Conversely, pharmacological or genetical blockade of Y_2 receptors has been shown to induce anxiolytic- and antidepressant-like profiles (Bacchi et al., 2006; Carvajal et al., 2006; Redrobe et al., 2003; Tschenett et al., 2003). Among the other NPY receptors, the Y_5 receptor subtype has been mainly studied in relation to food intake (Schaffhauser et al., 1997; Kask et al., 2001), however, based on its distribution in brain regions known for their role in emotional disorders (Dumont et al., 1998; Gerald et al., 1996), Y_5 has been suggested to be involved in the regulation of anxiety-like state and in the response to stressful stimuli (Sajdyk et al., 2002; Sørensen et al., 2004). The remaining receptor subtypes were not considered in this study; the Y_3 receptor subtype has not yet been cloned (Lee and Miller, 1998), while the Y_4 receptor was primarily found in peripheral tissues (Barrios et al., 1999). The Y_6 receptor gene was found to be completely absent in the rat (Burkhoff et al., 1998) and to be functional only in some species but not in human and other primates (Gregor et al., 1996; Matsumoto et al., 1996), whereas the Y_7 receptor has been cloned only in fish, amphibians and chicken (Fredriksson et al., 2004; Bromée et al., 2006).

Therefore, the present study was focused on the characterization of the three major NPY receptors—Y_1, Y_2 and Y_5—in order to understand their involvement in the regulation of the NPY role in the emotional states. This was investigated using a validated stress-induced animal model of depression, the chronic psychosocial stress, an ethological stress paradigm inducing depressive-like symptoms (Fuchs et al., 1996, 2001; Kramer et al., 1999) and evaluating the effects of the established antidepressant fluoxetine to counteract the alterations induced by stress. Two different animal species were investigated, a rodent (rat) and a pre-primate (tree shrew), the latter phylogenetically classified as an intermediate between insectivores and primates (Martin, 1990). In addition, since contrasting evidence about the receptor subtype mainly involved in the mediation of the role of NPY in the regulation of emotions still exists, the potential changes of the different receptor subtypes were also considered, in order to identify the receptor subtype primarily involved in the regulation of emotional processes.

2. Materials and methods

2.1. Animal care and treatments

Tissues from adult male rats and tree shrews were obtained from cohorts submitted to social conflict paradigm at the German Primate Center (Göttingen, Germany).

The experimental design of the study, the behavioural and hormonal characterisations of the rats used in the present study were previously described in detail (Rygula et al., 2006; Fig. 1). The rats used in the present study are the same as those included in the work of Rygula and colleagues. Three different experimental phases and four groups of animals ($n = 6$ rats per group) were generated: i) *Control*, ii) *Stress*, iii) *Control + fluoxetine*, iv) *Stress + fluoxetine*. The first experimental phase lasted for 7 days, during which all the animals were handled daily and body weight was recorded. The second phase also lasted for 7 days, during which the Wistar rats (intruders) of the *Stress* and *Stress + fluoxetine* groups were exposed daily to 1 h of psychosocial stress. The third experimental phase, lasting 28 days, consisted of the antidepressant treatment: stressed rats were maintained in the psychosocial conflict situation and were treated daily with fluoxetine or vehicle. Animals in the *Control + fluoxetine* and *Stress + fluoxetine* groups received fluoxetine (10 mg/kg body weight per day) orally directly before or after the defeat sessions. The drug (Fluoxetin Ratiopharm®, 4 mg/ml oral solution; Ratiopharm GmbH, Ulm, Germany) was administered using a bulb-headed cannula into the buccal cavity to minimize uncontrollable stress effects caused by daily injections. This dose has been demonstrated to be effective in reversing a series of endocrine and behavioural parameters modified by stress exposure (Rygula et al., 2006) and resulting with blood concentration of fluoxetine and its major active metabolite, norfluoxetine, similar to those reported in human patients treated with therapeutically active doses (Czéh et al., 2006). The animals of the *Control* and *Stress* groups were treated with vehicle only. On the last experimental day (day 42), all the animals were sacrificed, the brains were rapidly

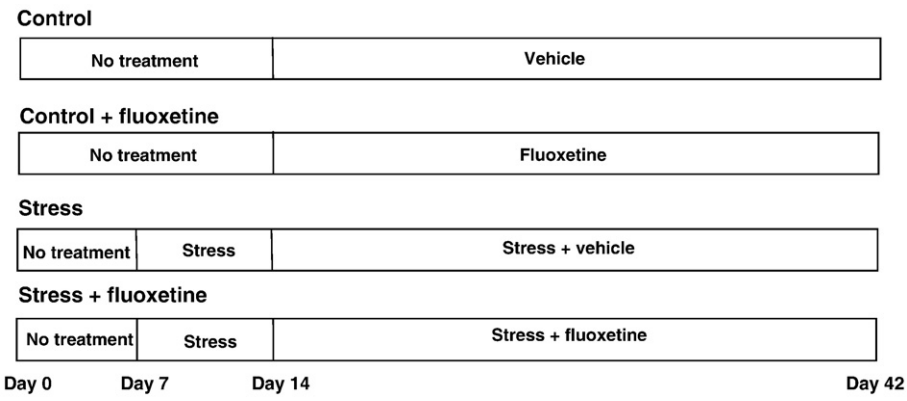

Fig. 1. Experimental groups and design of the study. For details, see the Materials and methods section and refer to Rygula et al. (2006).

removed, immediately frozen over liquid nitrogen, stored at −80 °C and sent to GlaxoSmithKline laboratories for *in vitro* assays.

The experimental design of the study on the tree shrews has been previously reported (Czéh et al., 2006). The experimental protocol was similar to that used in the rat model, with the difference that the animals were 4 per group and the daily administered dose of fluoxetine was 15 mg/kg. This dose has been demonstrated to be effective in restoring the hormonal, behavioural and morphological alterations induced by the exposure to stress in a previous study and resulting with serum concentration of fluoxetine and its major active metabolite close to the range reported for patients under fluoxetine treatment (Czéh et al., 2006). The behavioural and endocrine characterization of these animals after exposure to stress corresponded to that described by Fuchs (2005) and fluoxetine was able to counteract the effects of stress.

Animal experiments were conducted according to the European Council Directive of November 24, 1986 (86/609/EEC), and were approved by the Lower Saxony Federal State Office for Consumer Protection and Food Safety, Germany. The minimum number of animals required to obtain consistent data was used.

2.2. Tissue samples

Coronal sections (14 µm thick) were cut from the entire brains using a CM3050S cryostat (Leica), approximately at +1.60 mm, −1.88 mm, −2.30 mm and −3.14 mm from Bregma (Paxinos and Watson, 1998). The corresponding levels in the tree shrew brain (Tigges and Shantha, 1969) were also cut. The slices were thaw-mounted onto polarized SuperFrost Plus slides and stored at −80 °C until usage.

2.3. Riboprobes preparation

The riboprobes corresponding to rat NPY, Y_1 and Y_2 receptors mRNA were generated as previously described (Zambello et al., 2008). The rat Y_5 receptor riboprobe was made from a 600 bp cDNA of the entire Y_5 mRNA sequence (NM_012869), spanning from the amino acids 703–1302 of the receptor and then subcloned in a pCRII-TOPO vector (invitrogen). The probe specificities were evaluated with the public domain program Basic Local Alignment Search Tool (BLAST) in the NCBI package. No sequences displayed significant similarity with other sequences in the Non-Redundant database at NCBI. Rat riboprobes were also used for hybridizing tree shrew brain sections, given the high sequence homology existing between the two species (Fuchs and Flugge, 2002). Once subcloned in plasmid vectors, the sequences were linearized with appropriate restriction enzymes to generate antisense and sense riboprobes (Table 1). RNA probes complementary to the coding sequence were transcribed from the linearized plasmid templates with 2200 Ci/mM α-[^{33}P]UTP (Amersham Biosciences) using Sp6, T7 or T3 RNA polymerase. Transcriptions occurred in the presence of 100 mM 1,4-dithiothreitol, 0.5 mM each of ATP, GTP, CTP and 1 µg linearized plasmid template in 5× transcription buffer for 60 min at 37 °C. Then 1 µl DNase was added to the transcription cocktail, which was subsequently incubated for 10 min at 37 °C. The labelled riboprobes were then separated from unincorporated nucleotides using spin columns MicroSpin™ S-200 HR (Amersham Biosciences).

Table 1
Main steps used to obtain the rat riboprobes for NPY and its receptors Y_1, Y_2 and Y_5: length (in bp) of the cDNA fragments from which the riboprobes were generated; plasmid vectors in which the cDNA fragments were subcloned; restriction enzymes used to cut the cDNA fragments and RNA polymerase used to generate the sense and antisense riboprobes are reported.

	cDNA fragment	Plasmid vector	Restriction enzyme and RNA polymerase generating sense riboprobe	Restriction enzyme and RNA polymerase generating antisense riboprobe
Rat NPY	508 bp	pGEMZ4	DraI/T7	PstI/Sp6
Rat Y_1	245 bp	Bluescript II SK	PstI/T7	EcoRI/T3
Rat Y_2	423 bp	pBSKSII	BamHI/T3	XhoI/T7
Rat Y_5	600 bp	pCRII-TOPO	XhoI/Sp6	HindIII/T7

2.4. In situ hybridization

Tissue sections were warmed to room temperature and allowed to dry, then they were fixed in 4% formaldehyde/1× phosphate buffered saline (PBS) for 5 min, rinsed twice in 1× PBS and once in 0.1 M triethanolamine/0.9% sodium chloride pH 8 and then treated with 0.25% acetic anhydride/0.1 M triethanolamine/0.9% sodium chloride for 10 min. The sections were then rinsed in 2× saline sodium citrate (SSC), dehydrated in a graded series of ethanol (70%, 80%, 95%, 100%) and delipidated with chloroform. They were then allowed to air dry before being used or were frozen at −80 °C until use. All aqueous solutions used in the pre-hybridization phase were prepared with RNase-free water. The hybridization buffer consisted of 1 mg/ml sheared ssDNA, 500 µg/ml yeast tRNA, 2× Denhardt's solution, 20% dextran sulfate, 8× SSC and 50% formamide. Before the hybridization, the labelled probe was added to the hybridization mixture in the concentration of 2×10^4 cpm/µl, and 150 µl of this hybridization solution was applied to each slide. The sections of tissue were coverslipped to prevent evaporation and hybridization was carried out in a humified chamber overnight at 55 °C. Incubation was followed by washes in a graded series of SSC (2×, 1×, 0.5×, 0.1×), all at room temperature except for 0.1× SSC (53 °C) and dehydration was carried out with graded ethanol solutions (50%, 70%, 90%, 95%, 100%). The slides were then air dried and exposed to Fuji Imaging plates (BAS-TR 2025) together with ^{14}C standards for 4–5 days.

2.5. Quantification

The images from the *in situ* hybridization experiments were used for semi-quantitative analysis. Light transmittance values were measured from the digitalized images using an image analysis software system (AIS 4.0, Imaging Research, St. Catharines, Ontario, Canada). Based on the known radioactivity of the ^{14}C standards relative to their transmittance levels, the light transmittance values per mm^2 [PLS/mm^2] were converted to nCi/g using a calibration curve. All the regions of interest belong to the forebrain—i.e. cingulate cortex, septum, CA regions and dentate gyrus (DG) of hippocampus, amygdaloid and hypothalamic nuclei—and were chosen among those regions which reported changes in depression and stress-related disorders. They were defined by anatomical landmarks in conjunction with a rat brain atlas (Paxinos and Watson, 1998) and a tree shrew brain atlas (Tigges and Shantha, 1969). Generally, two consecutive sections were considered for each subject, based on the anatomy, and the regions were bilaterally analyzed and averaged. The measurements of each specific brain region were taken by individually tracing the structures on the TV monitor with a cursor.

2.6. Statistical analysis

Statistical evaluations of the differences among the experimental groups were assessed using "Statistica 6.0" software package. Data were analyzed by three-way ANOVA (analysis of variance), considering the pharmacological treatment (fluoxetine/vehicle), the stress exposure (stress/no stress) and the brain regions as factors and NPY or receptor mRNA expression as dependent variable. The ANOVA was followed by planned comparisons and Newman–Keuls *post-hoc* test. All the results were expressed as mean ± S.E.M. and the *p*-values were considered significant if lower than 0.05.

3. Results

3.1. NPY mRNA expression in the rat brain

The statistical analysis of NPY mRNA expression levels evaluated in the four experimental groups of rats revealed a significant difference in the NPY mRNA expression levels in the CA3 hippocampal region between *Control* and *Stress* group ($p = 0.011$), with lower levels in the stressed animals. In the DG of hippocampus, a significant effect of the interaction between exposure to chronic psychosocial stress and fluoxetine treatment was detected ($p = 0.018$), with a down-regulation in the *Stress* group of rats compared to the *Control* rats and an up-regulation in the *Stress + fluoxetine* group compared to the *Control + fluoxetine* group (Fig. 2A).

In all the other regions analyzed, cingulate cortex, septum, medial amygdala, CA1 and CA2 hippocampal regions and arcuate nucleus of hypothalamus, no significant changes were detected.

3.2. Y_1 receptor mRNA expression in the rat brain

A significant stress × treatment interaction was found in the Y_1 receptor mRNA expression levels in the ventro-medial hypothalamus (VMH; $p = 0.037$), in which a down-regulation was measured in the *Stress* group of rats compared to *Control* animals and an up-regulation was observed in the *Stress + fluoxetine* animals compared to the *Control + fluoxetine* group (Fig. 2B).

No significant differences were found in all the other regions considered (cingulate cortex, septum, central, medial and basolateral amygdala, paraventricular, and arcuate hypothalamic nuclei, CA1, CA2, CA3 and DG of hippocampus).

3.3. Y_2 receptor mRNA expression in the rat brain

A significant difference in Y_2 receptor mRNA expression levels was observed between *Control* and *Stress* rats in the ventro-medial hypothalamus, dorso-medial portion (VMHDM; $p = 0.027$), in which lower Y_2 receptor mRNA levels were found in the stressed rats compared to control rats (Fig. 2C).

There were no significant changes in the other regions considered: cingulate cortex, septum, central and medial amygdala, arcuate nucleus of hypothalamus, CA1, CA2, CA3 and DG of hippocampus.

3.4. Y_5 receptor mRNA expression in the rat brain

A significant effect of the interaction between exposure to psychosocial stress and fluoxetine treatment was observed in the VMH

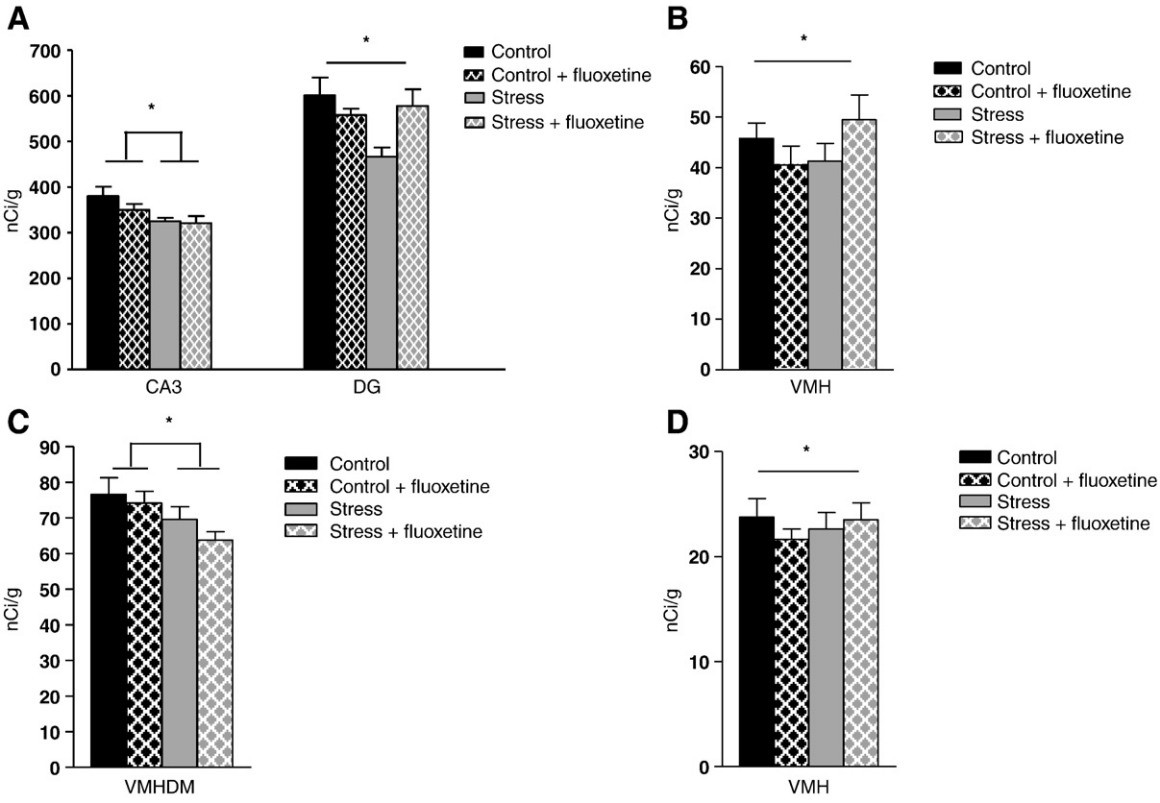

Fig. 2. NPY system mRNA levels detected in the brains of rats exposed to chronic psychosocial stress protocol. (A) NPY mRNA expression levels in the CA3 region and in the dentate gyrus (DG) of hippocampus of rats belonging to the *Control*, *Control + fluoxetine*, *Stress* and *Stress + fluoxetine* groups. The bar graph represents the mean ± S.E.M. ($n = 6$ rats/group) given as nCi/g (CA3 = 379.915 ± 21.12 in the *Control* group; CA3 = 349.925 ± 12.87 in the *Control + fluoxetine* group; CA3 = 324.98 ± 7.45 in the *Stress* group; CA3 = 320.97 ± 15.49 in the *Stress + fluoxetine* group. DG = 600.67 ± 39.7 in the *Control* rats; DG = 557.93 ± 14.04 in the *Control + fluoxetine* rats; DG = 466.32 ± 20.36 in the *Stress* rats e DG = 577.47 ± 37.11 in the *Stress + fluoxetine* rats). In the CA3 region: * $p < 0.05$: effect of stress. In the DG: * $p < 0.05$: effect of stress × treatment interaction. (B) Y_1 receptor mRNA expression levels in the ventro-medial hypothalamus (VHM) of rats belonging to the *Control*, *Control + fluoxetine*, *Stress* and *Stress + fluoxetine* groups. The bar graph represents the mean ± S.E.M. ($n = 6$ rats/group) given as nCi/g (VMH = 46.35 ± 2.0 in the *Control* rats; VMH = 40.97 ± 6.52 in the *Control + fluoxetine* rats; VMH = 41.98 ± 3.8 in the *Stress* rats and VMH = 48.31 ± 5.73 in the *Stress + fluoxetine* rats). * $p < 0.05$: effect of stress × treatment interaction. (C) Y_2 receptor mRNA expression levels in the ventro-medial hypothalamus, dorso-medial portion (VMHDM) of rats belonging to the *Control*, *Control + fluoxetine*, *Stress* and *Stress + fluoxetine* groups. The bar graph represents the mean ± S.E.M. ($n = 6$ rats/group) given as nCi/g (VMHDM = 76.57 ± 4.69 in the *Control* rats; VMHDM = 74.16 ± 3.3 in the *Control + fluoxetine* rats; VMHDM = 69.6 ± 3.58 in the *Stress* rats and VMHDM = 63.74 ± 2.37 in the *Stress + fluoxetine* rats). * $p < 0.05$: effect of stress. (D) Y_5 receptor mRNA expression levels in the ventro-medial hypothalamus (VHM) of rats belonging to the *Control*, *Control + fluoxetine*, *Stress* and *Stress + fluoxetine* groups. The bar graph represents the mean ± S.E.M. ($n = 6$ rats/group) given as nCi/g (VMH = 23.75 ± 1.77 in the *Control* rats; VMH = 21.64 ± 0.97 in the *Control + fluoxetine* rats; VMH = 22.62 ± 1.56 in the *Stress* rats and VMH = 23.5 ± 1.62 in the *Stress + fluoxetine* rats). * $p < 0.05$: effect of stress × treatment interaction.

($p=0.041$), in which a significant down-regulation of Y_5 receptor mRNA expression levels was measured in the rats of the *Stress* group compared to the *Control* rats, while an up-regulation was found in the *Stress + fluoxetine* group compared to the *Control + fluoxetine* (Fig. 2D).

All the other regions analyzed (cingulate cortex, septum, central, medial and basolateral amygdala, paraventricular and arcuate hypothalamic nuclei, hippocampal CA1, CA2, CA3 regions and DG) did not reveal significant variations of Y_5 receptor mRNA expression.

3.5. NPY mRNA expression in the tree shrew brain

The distribution of NPY and Y_1, Y_2, Y_5 receptor mRNA obtained in the tree shrew brain using riboprobes complementary to rat mRNA sequences was very similar to the distribution of the NPY system in the rat brain (Fig. 3).

The statistical analysis of the NPY mRNA expression levels evaluated in the four experimental groups of animals showed a significant difference in the NPY mRNA levels between *Control* and *Stress* animals, with higher NPY mRNA expression levels in the CA1 ($p=0.007$) and CA2 ($p=0.034$) hippocampal regions and in the arcuate nucleus of the hypothalamus ($p=0.01$) after exposure to psychosocial stress (Fig. 4A). In addition, the *post-hoc* analysis (Newman–Keuls test) showed a trend in the CA1 hippocampal region between *Control* and *Stress* groups of animals ($p=0.056$), between *Control* and *Stress + fluoxetine* groups ($p=0.092$) and between *Control + fluoxetine* and *Stress* groups ($p=0.083$). The same analysis conducted for the CA2 hippocampal region evidenced a trend between *Control* and *Stress* groups ($p=0.068$), between *Control + fluoxetine* and *Stress* groups ($p=0.051$) and between *Stress* and *Stress + fluoxetine* groups ($p=0.058$). Additionally, in the arcuate hypothalamic nucleus, the *post-hoc* analysis evidenced a significant interaction between *Control* and *Stress* groups ($p=0.047$) and between *Control + fluoxetine* and *Stress* groups ($p=0.027$). It is of great interest to mention that in all these regions, in particular in the arcuate hypothalamic nucleus, fluoxetine counteracted the effect of the chronic psychosocial stress by inducing a reduction of the NPY mRNA levels in the *Stress + fluoxetine* group and restoring the initial conditions.

No significant differences were found in all the other regions analyzed, such as cingulate cortex, septum, medial amygdala, CA3 region and DG of hippocampus.

3.6. Y_1 receptor mRNA expression in the tree shrew brain

The statistical analysis did not evidence any alteration in the tree shrew Y_1 receptor mRNA expression levels after exposure to stress, or induced by treatment with fluoxetine or due to the interaction between stress and fluoxetine in all the regions considered: cingulate cortex, septum, medial and basolateral amygdala, CA1, CA2, CA3 regions and DG of hippocampus.

3.7. Y_2 receptor mRNA expression in the tree shrew brain

The Y_2 receptor mRNA expression reported significant differences between *Stress* and *Control* groups in the CA3 hippocampal region, with higher levels in the stressed rats compared to control ($p=0.043$; Fig. 4B).

In all the other regions considered, such as cingulate cortex, septum, medial and basolateral amygdala, CA1, CA2, CA3 regions and DG of hippocampus no significant changes were observed.

3.8. Y_5 receptor mRNA expression in the tree shrew brain

The statistical analysis of the Y_5 receptor mRNA expression in the tree shrew brain reported a significant effect of the treatment with fluoxetine in the cingulate cortex, in which lower Y_5 mRNA levels were observed in the fluoxetine treated groups of animals compared to non-treated animals ($p=0.028$; Fig. 4C). In addition, in the same region a stress × treatment interaction was found, with an up-regulation measured in the *Stress* group of tree shrews compared to *Control* animals and a down-regulation in the *Stress + fluoxetine* group compared to *Control + fluoxetine* animals ($p=0.039$; Fig. 4C). Moreover, in the same region, the *post-hoc* analysis (Newman–Keuls test) evidenced a significant interaction in the Y_5 mRNA expression between *Stress* and *Stress + fluoxetine* groups ($p=0.027$), while a trend ($p=0.064$) was found between *Control* and *Stress* groups. Furthermore, a difference due to the interaction between stress and fluoxetine was observed in the CA1 ($p=0.044$) and CA2 ($p=0.0031$) hippocampal regions, in which lower Y_5 mRNA levels were measured in the *Stress* group compared to *Control* and higher levels were found in the *Stress + fluoxetine* compared to *Control + fluoxetine* group (Fig. 4C).

No significant differences were measured in all the other regions analyzed, such as septum, medial and basolateral amygdala, CA3 and DG of hippocampus.

4. Discussion

The impact of stressful events on the development of pathologies of the brain has been widely investigated and the use of social conflict between members of the same species is a natural stressor leading to a variety of long-lasting physiological, behavioural and molecular changes (Bjorkqvist, 2001; Fuchs and Flugge, 2002). In particular, it can affect reward-related processes (Von Frijtag et al., 2000) and evoke anhedonia and motivational deficits (Rygula et al., 2005), as well as changes in neurotransmitter release in discrete brain areas (Fuchs and Flugge, 2002; Krugers et al., 1993; Isovich et al., 2001; McKittrick et al., 2000). Moreover, a number of studies have reported that these changes were reversed by treatment with antidepressant drugs (Fuchs et al., 1996, 2004; Kramer et al., 1999; Rygula et al., 2006), confirming that chronic psychosocial stress represents a validated animal model of depression. Based on these premises, the present study investigated potential transcriptional changes of the NPY system in two different animal species, a rodent and a pre-primate, chronically exposed to psychosocial stress and the possible effect of fluoxetine, an established antidepressant widely used in clinical protocols. The purpose of this study was to assess if the mechanism by which chronic psychosocial stress induces a depressive-like state is mediated by this neuropeptidergic system and if this system is involved in the mechanism of action of an antidepressant drug.

Considering the rat, the NPY mRNA levels were found to be significantly decreased in the DG and CA3 hippocampal region of the stressed animals compared to controls (Fig. 2A), in line with previous findings involving a variety of animal models of depression, both genetic and stress-induced (Bjørnebekk et al., 2006; Caberlotto et al., 1998; Husum and Mathé, 2002; Jiménez-Vasquez et al., 2007; Mathé et al., 1998) and in support of the hypothesis that reduced hippocampal NPY levels are strictly related to the depressive-like state of the animal (Heilig, 2004). In particular, in the DG of hippocampus, an opposite regulation between stress exposure and antidepressant treatment was observed, consisting of a down-regulation of NPY mRNA expression in the stressed rats, reversed by chronic administration of fluoxetine, which restores control NPY levels (Fig. 2A). This result gives further support for a role of the hippocampal NPY system in the regulation of the neurobiological circuitry of emotional processes (Heilig, 2004). The present findings showing reduced NPY mRNA expression in the hippocampal CA3 region and DG of stressed rats could be partly related to the functional impairment and loss of hippocampal GABAergic interneurons, which are NPY positive (Kóhler et al., 1986; Deller and Leranth, 1990), observed under extreme, prolonged stress exposure (McEwen and Magarinos, 1997).

The present study gave further support to the existence of an anatomical and functional link between hippocampus and hypothalamus. As previously described, NPY mRNA was modulated only in the

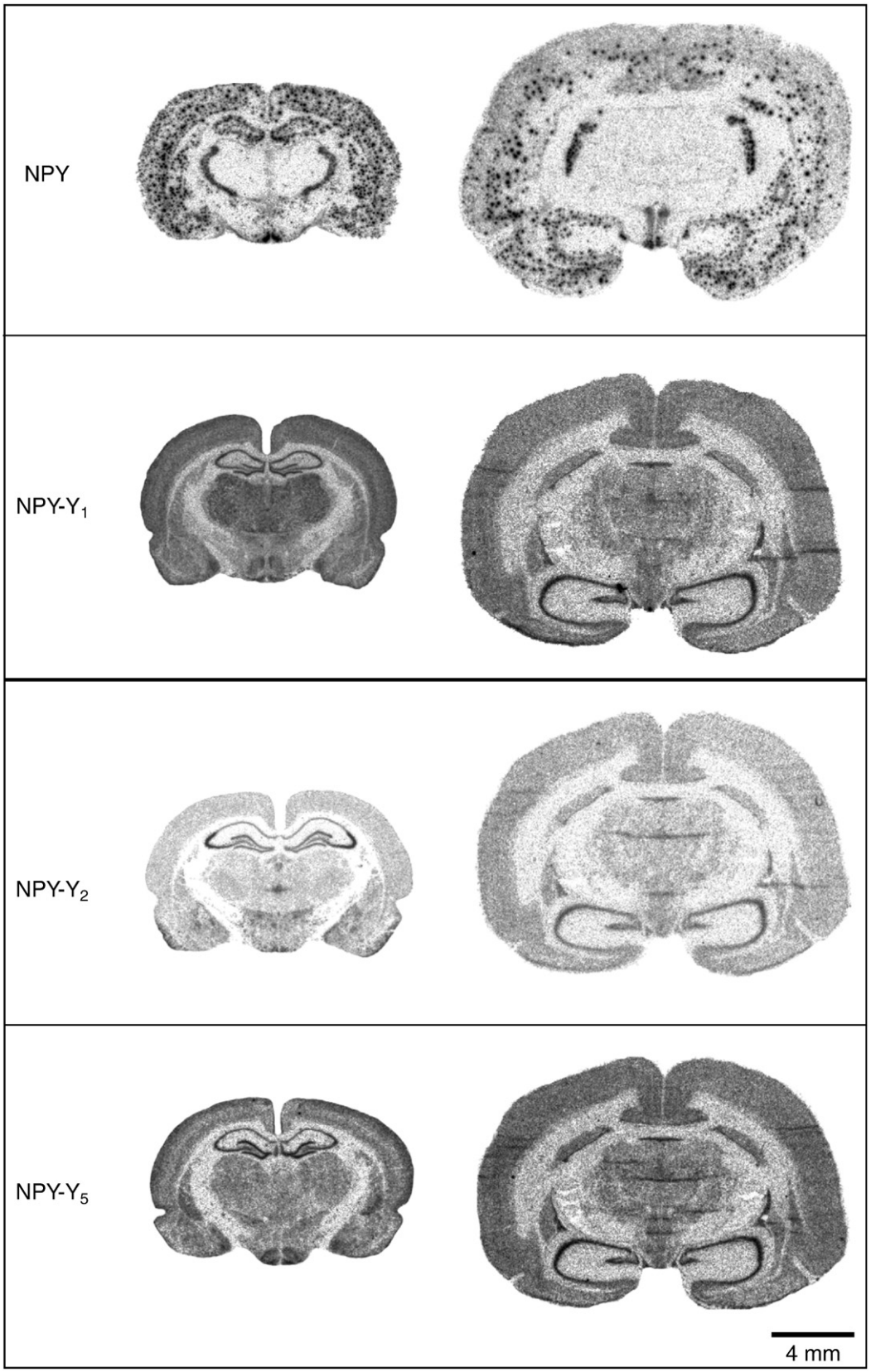

Fig. 3. Representative images of the distribution of NPY and its receptors Y_1, Y_2 and Y_5 mRNA expression in coronal sections of the rat brain (on the left), approximately at −3.14 mm from Bregma, and in the corresponding levels of the tree shrew brain (on the right). Scale bar = 4 mm.

hippocampus, whereas all the changes affecting NPY receptors were observed in hypothalamic nuclei. In particular, Y_1 and Y_5 receptors displayed a reciprocal regulation between stress exposure and fluoxetine treatment in the ventro-medial-hypothalamus (VHM), with reduced transcriptional levels in the stressed rats compared to controls and restored mRNA levels following fluoxetine treatment (Fig. 2B,D).

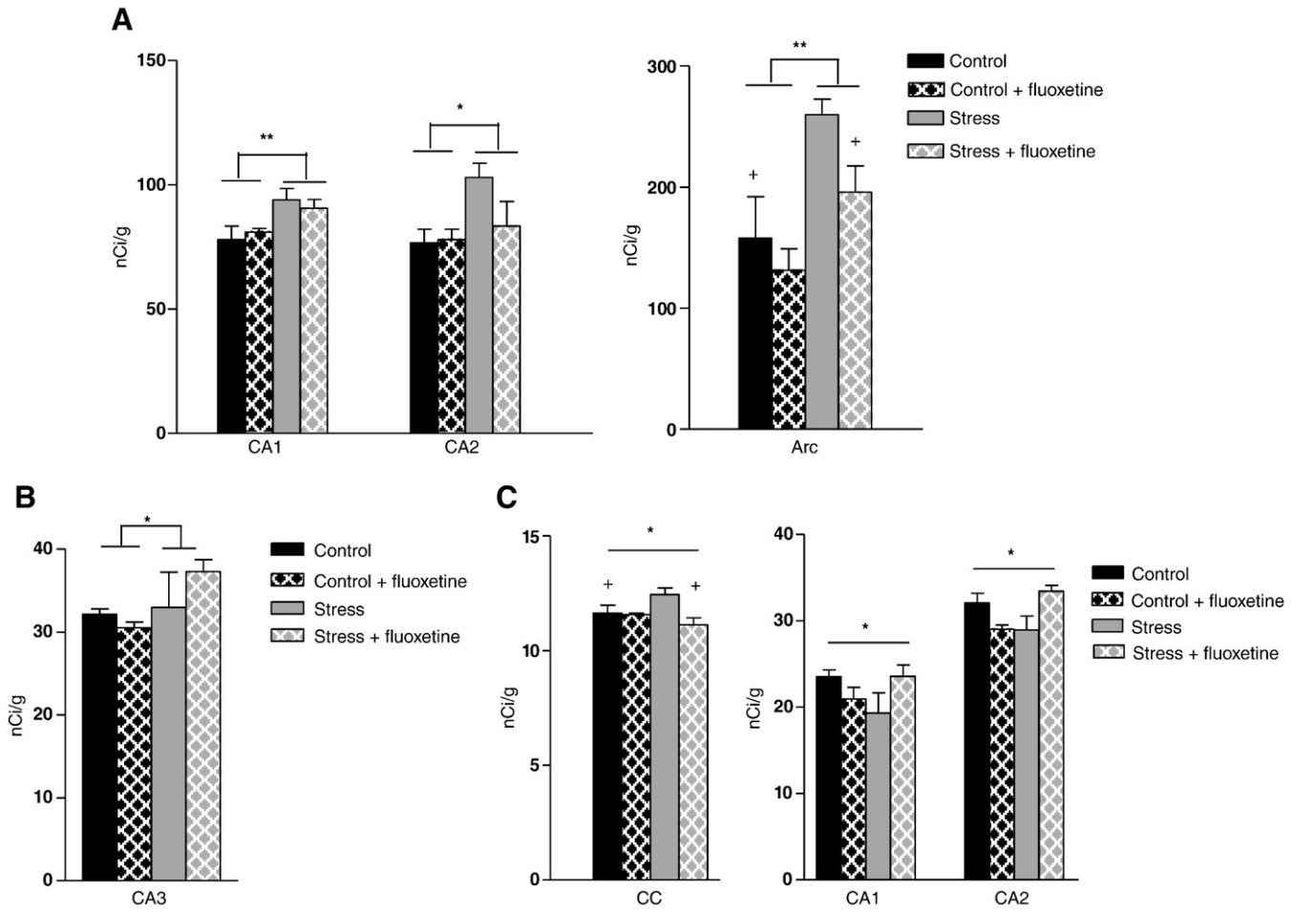

Fig. 4. NPY system mRNA levels detected in the brains of tree shrews exposed to chronic psychosocial stress protocol. (A) NPY mRNA expression levels in the CA1 and CA2 hippocampal regions (on the left) and in the arcuate nucleus (Arc) of hypothalamus (on the right) of tree shrews belonging to the *Control*, *Control +fluoxetine*, *Stress* and *Stress +fluoxetine* groups. The bar graph represents the mean ± S.E.M. ($n = 4$ animals/group) given as nCi/g (CA1 = 77.85 ± 5.5 in the animals of the *Control* group; CA1 = 80.93 ± 1.49 in the *Control +fluoxetine* group; CA1 = 93.99 ± 4.53 in the *Stress* group and CA1 = 90.57 ± 3.49 in the *Stress +fluoxetine* groups; CA2 = 76.61 ± 5.54 in the animals of the *Control* group; CA2 = 77.87 ± 4.19 in the *Control +fluoxetine* animals; CA2 = 102.96 ± 5.76 in the *Stress* group; CA2 = 83.37 ± 9.84 in the *Stress +fluoxetine* group; Arc = 157.83 ± 34.11 in the animals of the *Control* group; Arc = 131.15 ± 17.88 in the *Control +fluoxetine* group; Arc = 259.76 ± 12.85 in the *Stress* group; Arc = 195.83 ± 21.7 in the *Stress +fluoxetine* animals). In the CA1 region: ** $p<0.01$: effect of stress. In the CA2 region: * $p<0.05$: effect of stress. In the Arc: ** $p<0.01$: effect of stress; Newman–Keuls post-hoc test: $^{+}$ $p<0.05$ compared to *Stress* group. (B) Y_2 receptor mRNA expression levels in the CA3 hippocampal region of tree shrews belonging to the *Control*, *Control +fluoxetine*, *Stress* and *Stress +fluoxetine* groups. The bar graph represents the mean ± S.E.M. ($n = 4$ animals/group) given as nCi/g (CA3 = 32.15 ± 0.62 in the animals of the *Control* group; CA3 = 30.53 ± 0.67 in the *Control +fluoxetine* animals; CA3 = 32.98 ± 4.23 in the *Stress* group; CA3 = 37.27 ± 1.46 in the *Stress +fluoxetine* animals). * $p<0.05$: effect of stress. (C) Y_5 receptor mRNA expression levels in the cingulate cortex (CC, on the left), CA1 and CA2 hippocampal regions (on the right) of tree shrews belonging to the *Control*, *Control +fluoxetine*, *Stress* and *Stress +fluoxetine* groups. The bar graph represents the mean ± S.E.M. ($n = 4$ animals/group) given as nCi/g (CC = 11.63 ± 0.33 in the *Control* group; CC = 11.56 ± 0.066 in the *Control +fluoxetine* group; CC = 12.44 ± 0.29 in the *Stress* group; CC = 11.12 ± 0.31 in the *Stress +fluoxetine* group; CA1 = 23.54 ± 0.76 in the *Control* group; CA1 = 20.96 ± 1.34 in the *Control +fluoxetine* animals; CA1 = 19.32 ± 2.34 in the *Stress* group; CA1 = 23.56 ± 1.3 in the animals of the *Stress +fluoxetine* group; CA2 = 32.06 ± 1.13 in the *Control* group; CA2 = 29.03 ± 0.48 in the *Control +fluoxetine* animals; CA2 = 28.94 ± 1.6 in the *Stress* group; CA2 = 33.42 ± 0.66 in the *Stress +fluoxetine* animals). In the CC: * $p<0.05$: effect of stress and stress x treatment interaction; Newman–Keuls post-hoc test: $^{+}$ $p<0.05$ compared to *Stress* group. In the CA1 and CA2 regions: * $p<0.05$: effect of stress × treatment interaction.

These changes in Y_1 and Y_5 receptors were consistent with the hypothesis of their anatomical and functional interactions. They have been demonstrated to co-localize in several rat brain regions (Wolak et al., 2003) and a high dimerization state of these two receptor subtypes has been observed (Dinger et al., 2003), suggesting that they potentially exert a reciprocal regulation (Gehlert, 2004; Gehlert et al., 2007; Lin, 2004). Moreover, unlike the other NPY receptor subtypes, Y_1 and Y_5 human genes were found in close proximity to each other and were derived from a common precursor, through a gene duplication event (Herzog et al., 1997). In addition, as previously observed (Caberlotto et al., 1998; Jiménez-Vasquez et al., 2007), the reduced Y_1 receptor mRNA levels in stressed animals confirmed the role of Y_1 receptor in modulating NPY functions in stress-related responses and depressive-like states.

A significant reduction of the Y_2 receptor transcriptional levels was observed in the ventro-medial hypothalamic nucleus, dorso-medial portion (VMHDM) of the stressed rats, compared to non-stressed (Fig. 2C), suggesting that the chronic psychosocial conflict could be linked to alterations of the hypothalamic-pituitary adrenocortical (HPA) axis (Fuchs et al., 2001; Kramer et al., 1999). Consistent with this hypothesis, a parallel investigation of physiological parameters measured in these rats showed that the exposure to chronic psychosocial stress induces an increase in adrenal weight (Rygula et al., 2006), indicative of the effects of stress (Sapolsky et al., 2000), in line with a previous study reporting increased adrenal weight following chronic mild unpredictable stress (Muscat and Willner, 1992). The increased adrenal weight observed in the stressed animals might reflect a hyper-activation of the HPA axis (Kramer et al., 1999; Fuchs et al., 2001), a dysregulation representing one of the best replicated findings in human depressed patients (Rubin et al., 1987), further confirming the validity of the model.

Although animal models of depression are prevalently focused on rodents, in order to better investigate human condition, the present

study considered the effects of the chronic psychosocial stress on the NPY system in a non-rodent species, the tree shrew (*Tupaia belangeri*). Tree shrews are phylogenetically regarded as an intermediate between insectivores and primates and show a high genetic homology with humans (Martin, 1990), a pronounced territoriality in male animals and a day-active life (Fuchs et al., 1996). Based on these characteristics, the effect of chronic psychosocial stress on tree shrews could represent another experimental paradigm to study the mechanisms of major depression (Fuchs et al., 1996). In fact, numerous behavioural and endocrine studies of the effects of chronic social stress in the tree shrew have supported this hypothesis, demonstrating that these animals show behaviours mirroring symptoms of human depressed patients and respond to chronic antidepressant treatments, leading to an improvement of the symptoms (Fuchs, 2005; Fuchs and Flugge, 2002; Fuchs et al., 1996).

In contrast to the results in rats, chronic exposure to psychosocial stress in the tree shrews induced an increase of NPY mRNA levels in the CA1-2 hippocampal regions and in the arcuate hypothalamic nucleus (Fig. 4A). Although it was not easy to find an explanation to clarify the contrasting results in the two species, the observed up-regulation of NPY mRNA in the tree shrew could be due to a response of the NPY system to the stress-induced reduction of hippocampal neurogenesis in the DG observed in chronically stressed animals in parallel studies (Czéh et al., 2006, 2001; Fuchs et al., 2001). Indeed, the transcriptional up-regulation, particularly in the hippocampal formation, could be explained by the hypothesis of a neuroproliferative role of NPY, which counteracts the effect of stress in the hippocampal cells. Indeed, NPY has been shown to contribute to the maintenance of the hippocampal neurogenesis, thus suggesting a possible neuroprotective function against stress-induced cellular damage in the hippocampus (Howell et al., 2003, 2005, 2007). It could be speculated that NPY exerts its antidepressant properties and its improvement of learning and memory processing partly mediating its activity on hippocampal neuroproliferation.

The Y_2 receptor mRNA expression was increased in the CA3 hippocampal region of the stressed tree shrews compared to the non-stressed animals (Fig. 4B), confirming the suggested relevant role of Y_2 receptor in the regulation of emotional behaviour, supported by an increase of Y_2 mRNA in human post-mortem brains of suicides (Caberlotto and Hurd, 2001).

The NPY Y_5 receptor mRNA expression was found to be altered in various brain regions of the tree shrews, with a reduction of the transcriptional levels in the CA1-2 hippocampal regions of stressed animals, recovering the levels of the non-stressed animals after treatment with fluoxetine (Fig. 4C), as previously described for rats. Stress exposure also increased Y_5 receptor mRNA levels in other limbic regions, such as medial amygdala and cingulate cortex. The Y_5 receptor has been prevalently studied for its involvement in the mechanisms related to food intake and obesity (Gehlert, 1999; Cabrele et al., 2000). The results presented here are particularly interesting, since they represent the first example of Y_5 mRNA alteration in an animal model of depression and provide evidence for its involvement in the mechanism regulating the response to stress.

In conclusion, this study underlines that the NPY system transcriptional levels are affected by chronic psychosocial stress both in rat and tree shrew and demonstrated the ability of fluoxetine in preventing the effect of stress, by restoring the NPY system mRNA to control levels. In addition, these findings demonstrated that the three major NPY receptors, Y_1, Y_2 and Y_5 could have a role in modulating the NPY response to stress and this could be considered for a potential therapeutic treatment.

Finally, the results of this work have highlighted the existence of species-specific molecular mechanisms of response to stress and to the antidepressant treatment, which suggest the need of evaluating behavioural models in different animal species for a better understanding of psychiatric disorders.

References

Bacchi F, Mathé AA, Jimenez P, Stasi L, Arban R, Gerrard P, et al. Anxiolytic-like effect of the selective neuropeptide Y Y2 receptor antagonist BIIE0246 in the elevated plus-maze. Peptides 2006;27:3202–7.

Barrios VE, Sun J, Douglass J, Toombs CF. Evidence of a specific pancreatic polypeptide receptor in rat arterial smooth muscle. Peptides 1999;20:1107–13.

Bjorkqvist K. Social defeat as a stressor in humans. Physiol Behav 2001;73:435–42.

Bjørnebekk A, Mathé AA, Brene S. Running has differential effects on NPY, opiates, and cell proliferation in an animal model of depression and controls. Neuropsychopharmacology 2006;31:256–64.

Bromée T, Sjödin P, Fredriksson R, Boswell T, Larsson TA, Salaneck E, et al. Neuropeptide Y-family receptors Y6 and Y7 in chicken. Cloning, pharmacological characterization, tissue distribution and conserved synteny with human chromosome region. FEBS 2006;273:2048–63.

Burkhoff A, Linemeyer DL, Salon JA. Distribution of a novel hypothalamic neuropeptide Y receptor gene and it's absence in rat. Brain Res Mol Brain Res 1998;53:311–6.

Buwalda B, de Boer SF, Schmidt ED, Felszeghy K, Nyakas C, Sgoifo A, et al. Long-lasting deficient dexamethasone suppression of hypothalamic-pituitary-adrenocortical activation following peripheral CRF challenge in socially defeated rats. J Neuroendocrinol 1999;11:513–20.

Buwalda B, Felszeghy K, Horvath KM, Nyakas C, de Boer SF, Bohus B, et al. Temporal and spatial dynamics of corticosteroid receptor down-regulation in rat brain following social defeat. Physiol Behav 2001;72:349–54.

Caberlotto L, Hurd YL. Y(1) and Y(2) receptor mRNA expression in the prefrontal cortex of psychiatric subjects. Relationship of Y(2) subtype to suicidal behaviour. Neuropsychopharmacology 2001;25:91–7.

Caberlotto L, Fuxe K, Overstreet DH, Gerrard P, Hurd YL. Alterations in neuropeptide Y and Y1 receptor mRNA expression in brains from an animal model of depression: region specific adaptation after fluoxetine treatment. Brain Res Mol Brain Res 1998;59: 58–65.

Caberlotto L, Jimenez P, Overstreet DH, Hurd YL, Mathé AA, Fuxe K. Alterations in neuropeptide Y levels and Y1 binding sites in the Flinders Sensitive Line rats, a genetic animal model of depression. Neurosci Lett 1999;265:191–4.

Cabrele C, Langer M, Bader R, Wieland HA, Doods HN, Zerbe O, et al. The first selective agonist for the neuropeptide YY5 receptor increases food intake in rats. J Biol Chem 2000;275:36043–8.

Carvajal C, Dumont Y, Herzog H, Quirion R. Emotional behaviour in aged neuropeptide Y (NPY) Y2 knockout mice. J Mol Neurosci 2006;28:239–45.

Castagné V, Corder R, Gaillard R, Mormède P. Stress induced changes of circulating neuropeptide Y in the rat: comparison with catecholamines. Regul Pept 1987;19: 55–63.

Colmers WF, Wahlestedt C. The biology of Neuropeptide Y and related peptides. Totowa: Humana Press Inc.; 1993.

Czéh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, et al. Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci USA 2001;98:12796–801.

Czéh B, Simon M, Schmelting B, Hiemke C, Fuchs E. Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology 2006;31:1616–26.

Deller T, Leranth C. Synaptic connections of neuropeptide Y(NPY) immunoreactive neurons in the hilar area of the rat hippocampus. J Comp Neurol 1990;300:433–47.

Dinger MC, Bader JE, Kobor AD, Kretzschmar AK, Beck-Sickinger AG. Homodimerization of neuropeptide Y receptors investigated by fluorescence resonance energy transfer in living cells. J Biol Chem 2003;278:10562–71.

Dumont Y, Fournier A, Quirion R. Expression and characterization of the neuropeptide Y Y5 receptor subtype in the rat brain. J Neurosci 1998;18:5565–74.

Engler H, Dawils L, Hoves S, Kurth S, Stevenson JR, Schauenstein K, et al. Effects of social stress on blood leukocyte distribution: the role of alpha- and beta-adrenergic mechanisms. J Neuroimmunol 2004;156:153–62.

Fredriksson R, Larson ET, Yan YL, Postlethwait JH, Larhammar D. Novel neuropeptide Y Y2-like receptor subtype in zebrafish and frogs supports early vertebrate chromosome duplications. J Mol Evol 2004;58:106–14.

Fuchs E. Social stress in tree shrews as an animal model of depression: an example of a behavioural model of a CNS disorder. CNS Spectr 2005;10:182–90.

Fuchs E, Flugge G. Social stress in tree shrews: effects on physiology, brain function, and behaviour of subordinate individuals. Pharmacol Biochem Behav 2002;73: 247–58.

Fuchs E, Kramer M, Hermes B, Netter P, Hiemke C. Psychosocial stress in tree shrews: clomipramine counteracts behavioural and endocrine changes. Pharmacol Biochem Behav 1996;54:219–28.

Fuchs E, Flugge G, Ohl F, Lucassen P, Vollmann-Honsdorf GK, Michaelis T. Psychosocial stress, glucocorticoids, and structural alterations in the tree shrew hippocampus. Physiol Behav 2001;73:285–91.

Fuchs E, Czeh B, Kole MH, Michaelis T, Lucassen PJ. Alterations of neuroplasticity in depression: the hippocampus and beyond. Eur Neuropsychopharmacol 2004;14 (Suppl 5):S481–90.

Gehlert DR. Role of hypothalamic neuropeptide Y infeeding and obesity. Neuropeptides 1999;33:329–38.

Gehlert DR. Introduction to the reviews on neuropeptide Y. Neuropeptides 2004;38: 135–40.

Gehlert DR, Schober DA, Morin M, Berglund MM. Co-expression of neuropeptide Y Y1 and Y5 receptors results in heterodimerization and altered functional properties. Biochem Pharmacol 2007;74:1652–64.

Gerald C, Walker MW, Criscione L, Gustafson EL, Batzl-Hartmann C, Smith KE, et al. A receptor subtype involved in neuropeptide-Y-induced food intake. Nature 1996;382: 168–71.

Gregor P, Feng Y, DeCarr LB, Cornfield LJ, McCaleb ML. Molecular characterization of a second mouse pancreatic polypeptide receptor and its inactivated human homologue. J Biol Chem 1996;271:27776–81.
Heilig M. The NPY system in stress, anxiety and depression. Neuropeptides 2004;38: 213–24.
Herzog H, Darby K, Ball H, Hort Y, Beck-Sickinger A, Shine J. Overlapping gene structure of the human neuropeptide Y receptor subtypes Y1 and Y5 suggests coordinate transcriptional regulation. Genomics 1997;41:315–9.
Howell OW, Scharfman HE, Herzog H, Sundstrom LE, Beck-Sickinger A, Gray WP. Neuropeptide Y is neuroproliferative for post-natal hippocampal precursor cells. J Neurochem 2003;86:646–59.
Howell OW, Doyle K, Goodman JH, Scharfman HE, Herzog H, Pringle A, et al. Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus. J Neurochem 2005;93:560–70.
Howell OW, Silva S, Scharfman HE, Sosunov AA, Zaben M, Shatya A, et al. Neuropeptide Y is important for basal and seizure-induced precursor cell proliferation in the hippocampus. Neurobiol Dis 2007;26:174–88.
Husum H, Mathé AA. Early life stress changes concentrations of neuropeptide Y and corticotropin-releasing hormone in adult rat brain. Lithium treatment modifies these changes. Neuropsychopharmacology 2002;27:756–64.
Husum H, Vasquez PA, Mathé AA. Changed concentrations of tachykinins and neuropeptide Y in brain of a rat model of depression: lithium treatment normalizes tachykinins. Neuropsychopharmacol 2001;24:183–91.
Husum H, Gruber SH, Bolwig TG, Mathé AA. Extracellular levels of NPY in the dorsal hippocampus of freely moving rats are markedly elevated following a single electroconvulsive stimulation, irrespective of anticonvulsive Y1 receptor blockade. Neuropeptides 2002;36:363–9.
Husum H, Wortwein G, Andersson W, Bolwig TG, Mathé AA. Gene–environment interaction affects substance P and neurokinin A in the entorhinal cortex and periaqueductal grey in a genetic animal model of depression: implications for the pathophysiology of depression. Int J Neuropsychopharmacol 2008;11:93-101.
Ishida H, Shirayama Y, Iwata M, Katayama S, Yamamoto A, Kawahara R, et al. Infusion of neuropeptide Y into CA3 region of hippocampus produces antidepressant-like effect via Y1 receptor. Hippocampus 2007;17:271–80.
Isovich E, Engelmann M, Landgraf R, Fuchs E. Social isolation after a single defeat reduces striatal dopamine transporter binding in rats. Eur J Neurosci 2001;13:1254–6.
Jiménez-Vasquez PA, Overstreet DH, Mathé AA. in male and female brains of Flinders Sensitive Line, a rat model of depression. Effects of electroconvulsive stimuli. J Psychiatr Res 2000a;34:405–12.
Jiménez-Vasquez PA, Salmi P, Ahlenius S, Mathé AA. in brains of the Flinders Sensitive Line rat, a model of depression. Effects of electroconvulsive stimuli and d-amphetamine on peptide concentrations and locomotion. Behav Brain Res 2000b;111:115–23.
Jiménez-Vasquez PA, Mathé AA, Thomas JD, Riley EP, Ehlers CL. Early maternal separation alters neuropeptide Y concentrations in selected brain regions in adult rats. Brain Res Dev Brain Res 2001;131:149–52.
Jiménez-Vasquez PA, Diaz-Cabiale Z, Caberlotto L, Bellido I, Overstreet D, Fuxe K, et al. Electroconvulsive stimuli selectively affect behaviour and neuropeptide Y (NPY) and NPY Y(1) receptor gene expressions in hippocampus and hypothalamus of Flinders Sensitive Line rat model of depression. Eur Neuropsychopharmacol 2007;17: 298–308.
Kask A, Vasar E, Heidmets LT, Allikmets L, Wikberg JE. Neuropeptide Y Y(5) receptor antagonist CGP71683A: the effects on food intake and anxiety-related behavior in the rat. Eur J Pharmacol 2001;414:215–24.
Kendler KS, Walters EE, Neale MC, Kessler RC, Heath AC, Eaves LJ. The structure of the genetic and environmental risk factors for six major psychiatric disorders in women. Phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism. Arch Gen Psychiatry 1995a;52:374–83.
Kendler KS, Walters EE, Truett KR, Heath AC, Neale MC, Martin NG, et al. A twin-family study of self-report symptoms of panic-phobia and somatization. Behav Genet 1995b;25:499–515.
Kendler KS, Hettema JM, Butera F, Gardner CO, Prescott CA. Life event dimensions of loss, humiliation, entrapment, and danger in the prediction of onsets of major depression and generalized anxiety. Arch Gen Psychiatry 2003;60:789–96.
Kessler RC. The effects of stressful life events on depression. Annu Rev Psychol 1997;48: 191–214.
Kessler RC, Price RH, Wortman CB. Social factors in psychopathology: stress, social support, and coping processes. Annu Rev Psychol 1985;36:531–72.
Koolhaas JM, De Boer SF, De Rutter AJ, Meerlo P, Sgoifo A. Social stress in rats and mice. Acta Physiol Scand Suppl 1997;640:69–72.
Kramer M, Hiemke C, Fuchs E. Chronic psychosocial stress and antidepressant treatment in tree shrews: time-dependent behavioural and endocrine effects. Neurosci Biobehav Rev 1999;23:937–47.
Krugers HJ, Koolhaas JM, Bohus B, Korf J. A single social stress-experience alters glutamate receptor-binding in rat hippocampal CA3 area. Neurosci Lett 1993;154: 73–7.
Kóhler C, Smialowska M, Eriksson LG, Chan-Palay V, Davies S. Origin of the neuropeptide Y innervation of the rat retrohippocampal region. Neurosci Lett 1986;65:287–92.
Lee CC, Miller RJ. Is there really an NPY Y3 receptor? Regul Pept 1998;75–76:71–8.
Lin S. NPY and Y receptors: lessons from transgenic and knockout models. Neuropeptides 2004;38:189–200.
Martin RD. Primate origin and evolution. London: Chapman & Hall; 1990.
Mathé AA, Jimenez PA, Theodorsson E, Stenfors C. Neuropeptide Y, neurokinin A and neurotensin in brain regions of Fawn Hooded "depressed", Wistar, and Sprague Dawley rats. Effects of electroconvulsive stimuli. Prog Neuropsychopharmacol Biol Psychiatry 1998;22:529–46.
Matsumoto M, Nomura T, Momoses K, Ikeda Y, Kondou Y, Akiho H, et al. Inactivation of a novel neuropeptide Y/peptide YY receptor gene in primate species. J Biol Chem 1996;271:27217–20.
McEwen BS, Magarinos AM. Stress effects on morphology and function of the hippocampus. Ann N Y Acad Sci 1997;821:271–84.
McGrady AV. Effects of psychological stress on male reproduction: a review. Arch Androl 1984;13:1–7.
McGuffin P, Katz R. The genetics of depression: current approaches. Br J Psychiatry 1989:18–26 Suppl.
McKittrick CR, Magarinos AM, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR. Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. Synapse 2000;36:85–94.
Meerlo P, De Boer SF, Koolhaas JM, Daan S, Van Den Hoofdakker RH. Changes in daily rhythms of body temperature and activity after a single social defeat in rats. Physiol Behav 1996;59:735–9.
Muscat R, Willner P. Suppression of sucrose drinking by chronic mild unpredictable stress: a methodological analysis. Neurosci Biobehav Rev 1992;16:507–17.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th edition. New York: Academic Press; 1998.
Primeaux SD, Wilson SP, Cusick MC, York DA, Wilson MA. Effects of altered amygdalar neuropeptide Y expression on anxiety-related behaviours. Neuropsychopharmacology 2005;30:1589–97.
Redrobe JP, Dumont Y, Fournier A, Quirion R. The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. Neuropsychopharmacology 2002;26:615–24.
Redrobe JP, Dumont Y, Herzog H, Quirion R. Neuropeptide Y (NPY) Y2 receptors mediate behaviour in two animal models of anxiety: evidence from Y2 receptor knockout mice. Behav Brain Res 2003;141:251–5.
Rubin RT, Poland RE, Lesser IM, Martin DJ. Neuroendocrine aspects of primary endogenous depression-IV. Pituitary–thyroid axis activity in patients and matched control subjects. Psychoneuroendocrinology 1987;12:333–47.
Ruis MA, te Brake JH, Buwalda B, De Boer SF, Meerlo P, Korte SM, et al. Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. Psychoneuroendocrinology 1999;24:285–300.
Rüther E. Depression, circadian rhythms and trimipramine. Drugs 1989;38:49–50.
Rybkin II, Zhou Y, Volaufova J, Smagin GN, Ryan DH, Harris RB. Effect of restraint stress on food intake and body weight is determined by time of day. Am J Physiol 1997;273: R1612–22.
Rygula R, Abumaria N, Flugge G, Fuchs E, Ruther E, Havemann-Reinecke U. Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav Brain Res 2005;162:127–34.
Rygula R, Abumaria N, Domenici E, Hiemke C, Fuchs E. Effects of fluoxetine on behavioural deficits evoked by chronic social stress in rats. Behav Brain Res 2006;174:188–92.
Sajdyk TJ, Vandergriff MG, Gehlert DR. Amygdalar neuropeptide Y Y1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. Eur J Pharmacol 1999;368:143–7.
Sajdyk TJ, Schober DA, Smiley DL, Gehlert DR. Neuropeptide Y–Y2 receptors mediate anxiety in the amygdala. Pharmacol Biochem Behav 2002;71:419–23.
Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000;21:55–89.
Schaffhauser AO, Stricker-Krongrad A, Brunner L, Cumin F, Gerald C, Whitebread S, et al. Inhibition of food intake by neuropeptide Y Y5 receptor antisense oligodeoxynucleotides. Diabetes 1997;46:1792–8.
Sgoifo A, Koolhaas J, De Boer S, Musso E, Stilli D, Buwalda B, et al. Social stress, autonomic neural activation, and cardiac activity in rats. Neurosci Biobehav Rev 1999;23:915–23.
Sørensen G, Lindberg C, Wortwein G, Bolwig TG, Woldbye DP. Differential roles for neuropeptide Y Y1 and Y5 receptors in anxiety and sedation. J Neurosci Res 2004;77: 723–9.
Stefanski V, Engler H. Effects of acute and chronic social stress on blood cellular immunity in rats. Physiol Behav 1998;64:733–41.
Tigges J, Shantha TR. A stereotaxic brain atlas of the tree shrew (*Tupaia glis*). Baltimore: Williams & Wilkins Co; 1969.
Tornatzky W, Miczek KA. Behavioural and autonomic responses to intermittent social stress: differential protection by clonidine and metoprolol. Psychopharmacology (Berl) 1994;116:346–56.
Tschenett A, Singewald N, Carli M, Balducci C, Salchner P, Vezzani A, et al. Reduced anxiety and improved stress coping ability in mice lacking NPY-Y2 receptors. Eur J Neurosci 2003;18:143–8.
van Erp AM, Kruk MR, Meelis W, Willekens-Bramer DC. Effect of environmental stressors on time course, variability and form of self-grooming in the rat: handling, social contact, defeat, novelty, restraint and fur moistening. Behav Brain Res 1994;65:47–55.
von Frijtag JC, Reijmers LG, Van der Harst JE, Leus IE, Van den BR, Spruijt BM. Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. Behav Brain Res 2000;117:137–46.
Wolak ML, DeJoseph MR, Cator AD, Mokashi AS, Brownfield MS, Urban JH. Comparative distribution of neuropeptide Y Y1 and Y5 receptors in the rat brain by using immunohistochemistry. J Comp Neurol 2003;464:285–311.
Wortwein G, Husum H, Andersson W, Bolwig TG, Mathé AA. Effects of maternal separation on neuropeptide Y and calcitonin gene-related peptide in "depressed" Flinders Sensitive Line rats: a study of gene–environment interactions. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:684–93.
Zambello E, Jiménez-Vasquez PA, El Khoury A, Mathé AA, Caberlotto L. Acute stress differentially affects corticotropin-releasing hormone mRNA expression in the central amygdala of the "depressed" flinders sensitive line and the control flinders resistant line rats. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:651–61.