ORIGINAL INVESTIGATION

Mesolimbic dopamine D_2 receptor plasticity contributes to stress resilience in rats subjected to chronic mild stress

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Abstract
Rationale Few studies have investigated neurobiological and biochemical differences between stress-resilient and stress-vulnerable experimental animals.
Objectives We investigated alterations in mesolimbic dopamine D_2 receptor density and mRNA expression level in stressed rats at two time points, i.e. after 2 and 5 weeks of chronic mild stress (CMS).
Methods We used the chronic mild stress paradigm because it is a well-established animal model of depression. Two groups of stressed rats were distinguished during CMS experiments: (1) stress reactive (70 %), which displayed a decrease in the drinking of a palatable sucrose solution during the stress regimen, and (2) stress resilient (30 %), which exhibited an unaltered drinking profile when compared with the unchallenged control group. [^{3}H]Domperidone was used as a ligand to label dopamine D_2 receptors, and a mixture of three specific oligonucleotides was used to evaluate dopamine D_2 receptor mRNA changes in various regions of the rat brain.
Results CMS strongly affected the mesolimbic dopamine circuit in stress-resilient group after 2 weeks and stress-reactive group of rats after 5 weeks which exhibited a decrease in the level of dopamine D_2 receptor protein without alterations in D_2 mRNA expression. Stress-resilient animals, but not stress-reactive animals, effectively adapted to the extended stress and coped with it. The increase in D_2 mRNA expression returned the dopamine D_2 receptor density to control levels in stress-resilient rats after 5 weeks of CMS, but not in stress-reactive animals.
Conclusions These results clearly demonstrate that, despite earlier blunting, the activation of dopamine receptor biosynthesis in the dopamine mesoaccumbens system in stress-resilient rats is involved in active coping with stressful experiences, and it exhibits a delay in time.

Keywords Chronic mild stress · Depression · Domperidone · D_2 receptor · Dopamine mesolimbic system · In situ hybridisation · mRNA · Stress resilience · Rat

Introduction

Psychological resilience among people in the face of trauma, adversity and chronic stress has received much attention since the 1970s (Feder et al. 2009). Many behavioural and psychological aspects have been investigated, and differences have been observed between stress-resilient and stress-vulnerable people. The stress-resilient group exhibits active coping in the face of trauma and an optimistic mood, and they cognitively reinterpret stressful situations and may possess a strong moral compass and life purpose (Haglund et al. 2007; Feder et al. 2009; Cicchetti 2010; Rowland 2011).

Advanced biological, molecular and brain imaging techniques as well as animal models of mental illnesses have allowed the delineation of multiple genetic, biochemical, neurological, neurochemical and neuroendocrine changes that define the stress-vulnerable phenotype. However, relatively few studies have examined differences in neurobiological systems among individuals who are "resistant" to stress. Therefore, further intensive study is required to define the molecular factors and aspects of resilience.

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Noradrenergic and serotonergic catecholamine circuits are imbalanced in stress-evoked depression. This phenomenon is well established, and these circuits are the main target of action of antidepressant drugs. These drugs immediately influence catecholamine concentrations, but their clinical and behavioural effects appear after several weeks of chronic administration. These two circuits are indirectly involved in mood control via ascending innervations that regulate the mesolimbic dopamine system (Willner et al. 2005; Dunlop and Nemeroff 2007). However, the dopaminergic system has received less attention regarding vulnerability and resilience to depression under different circumstances. The mesolimbic dopamine circuit, particularly dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens septi (NAcc), is the downstream terminal link in the nerve cascade that regulates reward- and aversion-related behaviours (Gershon et al. 2007; Krishnan et al. 2007). Stress exerts a strong influence on this system and elicits specific responses depending on the type and duration of aversive stimulation (Puglisi-Allegra et al. 1991; Cabib and Puglisi-Allegra 1994, 1996a, b; Finlay and Zigmond 1997). Therefore, impairment of the stress-induced mesolimbic dopamine system leads to the development of anhedonia, which is a core symptom of depression. The rapid antidepressant action of dopamine D_2 receptor agonists, such as pramipexole or bromocriptine, likely explains the direct involvement of the mesolimbic circuit and dopamine D_2 receptors in depression (Dailly et al. 2004). Alterations in dopamine concentration in the mesolimbic reward system and dopamine D_2 receptors in response to stress accompany the development of depression-like psychopathology (Imperato et al. 1993; Papp et al. 1994; Dziedzicka-Wasylewska et al. 1997; Larisch et al. 1997; Cabib et al. 1998; Yadid et al. 2001; Gershon et al. 2007). Dopamine concentrations in the NAcc are increased in response to and in expectation of reward or aversive stimuli, but a blunted response leads to anhedonia, which is a long-lasting effect of mild chronic stress (Di Chiara and Tanda 1997, Di Chiara et al. 1999; Dunlop and Nemeroff 2007). Our study used the chronic mild stress (CMS) paradigm, which is a well-characterised animal model of depression with good face and predictive and construct validity. The chronic exposure of rats to mild unpredictable stressors produces behavioural deficits (anhedonia) that may be maintained for several months, and chronic treatment with antidepressants restores normal behaviour in stressed animals (Willner et al. 1987; Papp et al. 1994; Bielajew et al. 2002; Bekris et al. 2005). Approximately 30 % of animals that are subjected to several weeks of CMS demonstrate stress resilience that is manifested as unaltered consumption of a palatable 1 % sucrose solution in this model (Bergstrom et al. Bergström et al. 2008). These animals are usually rejected from further analysis as "bad-responding" subjects that do not demonstrate the behavioural response. However, this group mimics

the behavioural response to stress that is observed in people who do not develop depression in the face of adversity. Therefore, the present study investigated differences in the dopaminergic mesolimbic system, especially dopamine D_2 receptor density and mRNA expression, in various brain regions between stress-reactive and stress-resilient rats which followed CMS paradigm. The novel approach in our study involves the follow-up of temporal changes in the dopamine circuit during the CMS paradigm in both groups of animals, after 2 and 5 weeks of the stress procedure. This study clearly demonstrates that resilient animals exhibit strong neuroadaptive abilities that allow them to dynamically and actively cope with stress and adversity.

Experimental procedures

Animals

Male Wistar Han rats (Charles River, Germany) were brought into the laboratory 1 month prior to the start of the behavioural and biochemical experiments and, with some exceptions described below, were singly housed in plastic cages ($40 \times 25 \times 15$ cm) with food and water provided ad libitum on a 12-h light/dark cycle under constant temperature (22 ± 2 °C) and humidity (50 ± 5 %). The animals weighed between 300 and 400 g at the end of the behavioural experiments.

Behavioural procedure and tissue preparation

Two CMS experiments were performed according to the method described by Papp et al. (1994). All animals ($n = 100$) were adapted to laboratory and housing conditions for 2 weeks and trained to a drink palatable sucrose solution (1 %). The training procedure lasted 6 weeks and consisted of 1-h testing sessions every week (at 10:00 AM on Tuesdays) in which the sucrose solution was presented to the rats in their home cages after 14 h of food and water deprivation. Sucrose intake was measured after each drinking test as differences in bottle weight. Ten animals were excluded from the experiment after the 6-week training procedure because of non-stable drinking profiles. The remaining animals ($n = 90$) were randomly divided into two groups and subjected to control ($n = 20$) or stress ($n = 70$) conditions. The stress regimen was initiated at week 6. Half of the stressed animals ($n = 35$) were subjected to the chronic mild stress procedure for 2 weeks, and the other half ($n = 35$) of the stressed animals underwent CMS for five consecutive weeks. The stress schedule included two periods of food or water deprivation, a 45° cage tilt, intermittent illumination, a soiled cage (200 ml of water in the sawdust bedding), paired housing, low-intensity stroboscopic illumination (150 flashes/min) and two periods of no stress. All stressors had a duration of 10–14 h and were applied individually and

continuously during light and dark periods. Control animals were remained undisturbed in a separate room with free access to food and water, except for a period of overnight deprivation for the sucrose consumption test once per week. Three different groups of animals were identified based on sucrose intake after 2 and 5 weeks of the CMS procedure:

– 2-week CMS: (1) control animals ($n=10$), (2) stress-reactive rats ($n=25$) and (3) stress-resilient rats ($n=10$);
– 5-week CMS: (1) control animals ($n=10$), (2) stress-reactive rats ($n=24$) and (3) stress-resilient rats ($n=11$).

Thirty percent of all rats that were subjected to the chronic mild stress procedure were stress resilient ($n=21$). Ten representative stress-reactive animals from the 2- and 5-week CMS were selected for biochemical analyses. The remaining stress-reactive animals ($n=29$) were used in another experiment, and these animals were not included in this study.

The rats were sacrificed by decapitation 24 h after the last sucrose test. The brains were rapidly removed and frozen using a heptane–dry ice mixture. Coronal brain sections (12 μm) through the nucleus accumbens septi, striatum and ventral tegmental area (Paxinos and Watson 1998) were cut using a Jung CM 3000 cryostat microtome (Leica, Germany). The slices were thaw mounted on gelatine-covered microscope slides, air dried and stored at −20 °C until use.

[^{3}H]Domperidone binding to dopamine D$_2$ receptors and analysis of autoradiograms

Tissue sections were pre-incubated in 50 mM Tris–HCl buffer (pH 7.4) at room temperature for 15 min to remove endogenous dopamine. The brain slices were incubated for 2 h at room temperature in 50 mM Tris–HCl (pH 7.4) incubation buffer containing 0.4 nM [^{3}H]domperidone and the following ions: 120 mM NaCl, 1 mM EDTA, 1.5 mM CaCl$_2$, 4 mM MgCl$_2$ and 5 mM KCl. [^{3}H]Domperidone concentrations corresponded to Kd value (Seeman et al. 2003). Parallel sections treated with 10 μM (+)butaclamol were incubated in the buffer described above to determine non-specific binding. Incubation was terminated by washing the sections twice in 50 mM Tris–HCl (pH 7.4) at 4 °C for 5 min and once in ice-cold distilled water. The sections were dried overnight under a stream of air. The labelled brain slices were placed against an imaging plate (Fujifilm, Japan) with autoradiographic microscales (GE Healthcare) for 7 days. The obtained autoradiograms were analysed and quantified using ImageGauge software (Fujifilm, Japan). The specific binding of radioligand to dopamine D$_2$ receptors was calculated by subtracting non-specific binding images in adjacent brain slices from the total binding signal. The results are expressed in femtomole of bound radioligand per milligram of tissue (fmol/mg) in each examined structure.

Dopamine D$_2$ receptor mRNA in situ hybridisation

The rat brain sections were fixed for 10 min in cold 4 % formaldehyde, briefly washed in PBS and incubated for 10 min in an ice-cold acetic anhydride (0.25 %)—TEA (0.1 M) solution—to minimise unspecific oligonucleotide hybridisation. The fixed brain slices were dehydrated in a graded series of alcohols and subjected to two 10-min incubations in chloroform to remove lipids. The prepared tissue slices were washed in ethanol and air dried. A mixture of three commercially available oligonucleotides complementary to 4–51, 766–813 and 848–901 bp of the rat dopamine D$_2$ receptor mRNA was used in the in situ hybridisation assays (Dziedzicka-Wasylewska et al. 1997; Bunzow et al. 1988). Oligonucleotide probes were labelled at the 3′ end with [^{35}S]dADP (Hartmann Analytic, Germany) using terminal transferase (Fermentas, Lithuania). The probes were suspended to a final concentration of $1×10^6$ disintegration per minute (dpm) per 50 μl of hybridisation buffer containing the following components: 50 % (v/v) formamide, 10 % (w/v) dextran sulphate, 4× saline-sodium citrate (SSC) (pH 7.0), 1× Denhardt's solution (0.02 % polyvinylpyrrolidone/0.02 % Ficoll/0.02 % bovine serum albumin), yeast tRNA (0.25 mg/ml), sheared herring sperm DNA (0.2 mg/ml) and 10 mM dithiotreitol. All solutions were prepared in deionised 0.1 % DEPC-treated water. Hybridisation buffer containing 50 μl of $1×10^6$ dpm of radiolabelled oligonucleotides was applied to each fixed and prehybridised slide, and the slides were covered by Parafilm and incubated for 18 h at 37 °C in humid conditions. Each slide was briefly washed twice in a 1× SSC solution at room temperature after hybridisation followed by four washes (15 min each) in 2× SSC with 50 % formamide at 42 °C and one for 15-min wash at room temperature in 1× SSC. The hybridised brain sections were rinsed with deionised water, dehydrated in increasing concentrations of ethanol and air dried. Prepared tissue sections were placed into X-ray cassettes and exposed to film plates (Kodak) for 20 days at a temperature of −20 °C. The developed autoradiograms were analysed, quantified and normalised using ImageGauge software (Fujifilm, Japan). Data are expressed as percent changes in mRNA signals compared with the control group (expressed as 100 %).

Statistical analysis of the data

The values from the CMS behavioural tests represent the mean sucrose intake at the corresponding time point. Two-way ANOVA and independent repeated measures ANOVA followed by Bonferroni's post hoc test were used to analyse the data from the 6 weeks of sucrose training and the 2 and 5 weeks of CMS. [^{3}H]Domperidone-binding data are expressed as the mean density of dopamine D$_2$ receptor protein per milligram of tissue [in femtomole per milligram ± standard error of the mean (SEM)] in each group. Data

from in situ hybridisation assays were normalised to the data of the control group (expressed as 100 %). These data were analysed using one-way ANOVA and Bonferroni's multiple comparison post hoc test by using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Sucrose intake

Independent analysis using repeated measures ANOVA test did not show any significance of sucrose consumption among animals during training procedure in 2- and 5-week CMS experiment ($F_{2, 29}=1.190$; $p=0.3271$, and $F_{2, 29}=3.485$; $p=0.0526$, respectively). After the CMS experiments, we were able to ascribe the animals retrospectively to the stress-resilient and stress-susceptible groups on the

basis of their individual drinking behaviour. Two-way ANOVA revealed significant effects of stress ($F_{14, 216}=17.51$; $p<0.001$), time ($F_{14, 216}=2.630$; $p<0.05$) and stress×time ($F_{14, 216}=2.531$; $p<0.005$) on sucrose consumption after 2 weeks of CMS. A significant effect of stress ($F_{20, 297}=44.46$; $p<0.0001$) and stress×time ($F_{20, 297}=2.303$; $p<0.005$) on sucrose consumption was observed after 5 weeks of CMS in the respective groups of rats. Repeated measures ANOVA revealed that the application of the stress procedure for 2 weeks allowed the selection of stress-reactive animals ($F_{2, 29}=14.74$; $p<0.005$) which exhibited a significant decrease in sucrose consumption as compared to non-stressed control group. A separate group of rats did not change their drinking behaviour when compared with control rats ($F_{2, 29}=14.74$; $p>0.05$) (Fig. 1a).

A similar response was observed in the 5-week CMS rats. A proportion of the animals responded to stress with a decrease in sucrose consumption ($F_{2, 29}=10.79$; $p<0.05$),

Fig. 1 The effect of exposure to chronic mild stress on the consumption of a 1 % sucrose solution. Control (*empty circle connected with straight lines*), stress-reactive (*filled square connected with bullet operators*) and stress-resilient (*filled circle connected with broken lines*) groups were selected. a Two weeks of CMS, b 5 weeks of CMS. Data represent the mean ± SEM, $n=10$; *$p<0.05$; **$p<0.01$; ****$p<0.0001$ vs. control group
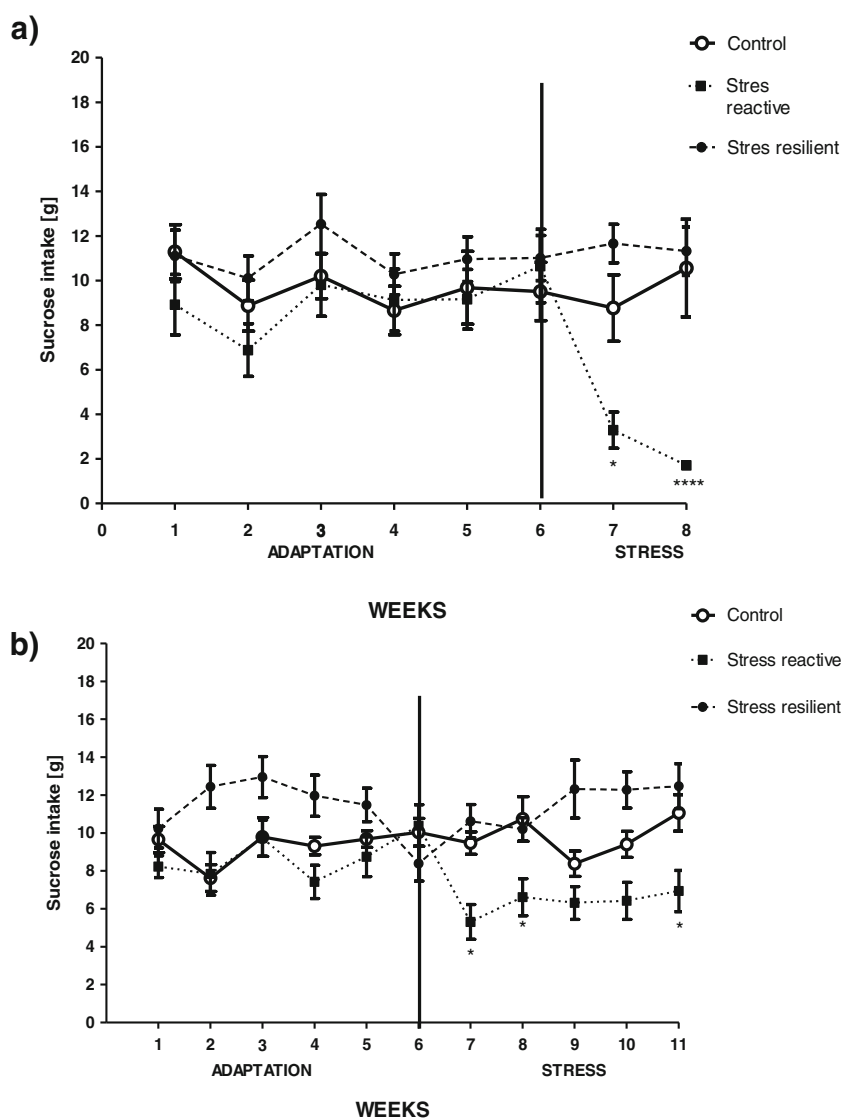
and other animals were unaffected by stress ($F_{2, 29}=10.79$; $p>0.05$) as compared to control rats (Fig. 1b). Our arbitrary retrospective observations have shown that anhedonic animals decreased their sucrose consumption to below 7.5 g when compared with the sucrose intake measurement performed at the end of the sucrose training period (i.e. sixth week).

Effect of CMS procedure on dopamine D$_2$ receptors in the rat brain—autoradiography and in situ hybridisation

Representative autoradiograms of the distribution of [³H] domperidone-binding sites and dopamine D$_2$ receptor mRNA in various regions of the rat brain are presented in Fig. 2. The impact of the CMS procedure on dopamine D$_2$ receptors was examined in several rat brain areas. Two weeks of CMS reduced [³H]domperidone binding in the striatum (medial and lateral), nucleus accumbens (shell and core) and the lateral, but not medial, ventral tegmental area. However, this reduction only reached statistical significance in animals that were resilient to CMS procedure (Fig. 3a–f).

Two weeks of CMS did not alter dopamine D$_2$ receptor mRNA expression in the examined brain regions (Fig. 4). Five weeks of the CMS procedure reduced [³H]domperidone binding to dopamine D$_2$ receptors in the striatum (lateral and medial) and nucleus accumbens (core) in stress-responsive rats. No significant differences in [³H] domperidone binding were observed in the brains of stress-resilient animals (Fig. 5a, b, d). However, the reduction in [³H]domperidone binding in the nucleus accumbens

shell was observed in both stress-reactive and stress-resilient rats (Fig. 5c) No significant differences in [³H]domperidone binding were observed in the ventral tegmental area when compared with the control group. However, a statistically significant difference was observed in dopamine D$_2$ receptor density between stress-reactive and stress-resilient animals in the medial part of the VTA (Fig. 5e, f).

In situ hybridisation revealed a significant increase in dopamine D$_2$ receptor mRNA in the striatum (lateral and medial), nucleus accumbens (shell) and ventral tegmental area (lateral and medial) in stress-resilient rats (Fig. 6a–f). No significant differences were observed in stress-reactive animals.

Discussion

Recent research has focused on the mechanisms that underlie the susceptibility to stress (Larish et al. Larisch et al. 1997; Husseini et al. 2001; Zhu et al. 2011; Blugeot et al. 2011). However, only a small percentage of people who face adverse events develop depression (Feder et al. 2009; Cicchetti 2010). Therefore, the factors that contribute to stress resilience may be more worthy of investigation. The present study examined stress-induced changes in mesolimbic dopamine D$_2$ receptor protein and mRNA using chronic mild stress at two time points (after 2 and 5 weeks of CMS) in two groups of animals with different behavioural responses to stress.

The CMS paradigm is a well-established animal behavioural model of anhedonia, which is a core symptom of

Fig. 2 Representative autoradiograms of specific [3H] domperidone binding **a**, **b** and dopamine D$_2$ receptor mRNA **c**, **d** in coronal sections of the rat brain. The brain regions used for quantitative analysis were chosen according to Paxinos and Watson (1998) **e**, **f**; *Bregma*, 2.28–1.8 mm **a**, **c** and (−5.04) to (−5.28)mm **b**, **d**
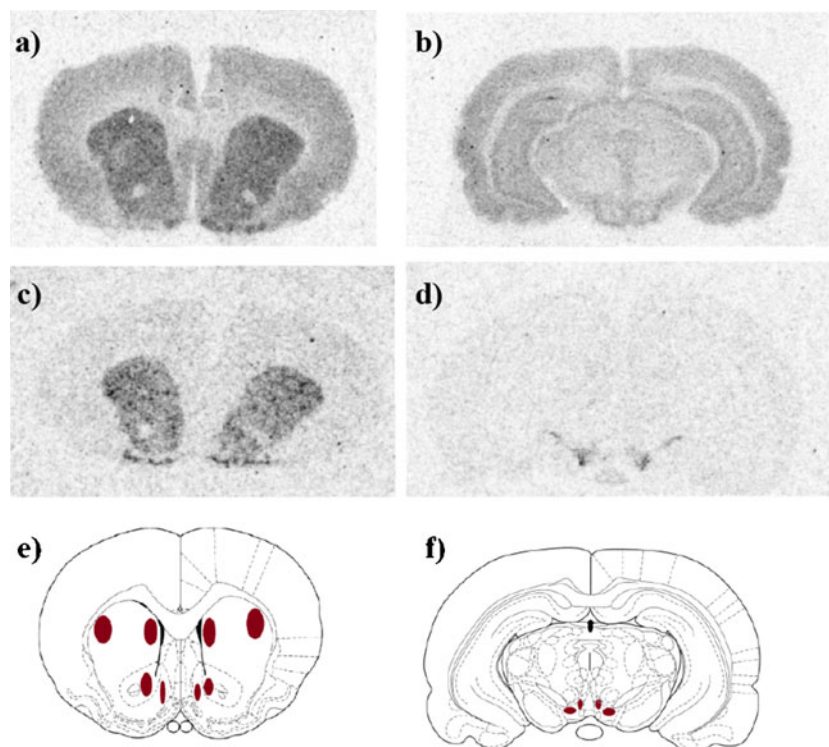
Fig. 3 The effect of 2 weeks of chronic mild stress on specific [3H]domperidone binding in various regions of the rat brain. **a** Lateral striatum; **b** medial striatum; **c** nucleus accumbens septi, core; **d** nucleus accumbens septi, shell; **e** lateral ventral tegmental area; **f** medial ventral tegmental area. Data represent the means ± SEM; $n=$ 10 (fmol/mg tissue); ANOVA followed by Bonferroni's multiple comparison post hoc test; $*p<0.05$; $**p<0.01$; $***p<0.001$; $****p<0.0001$
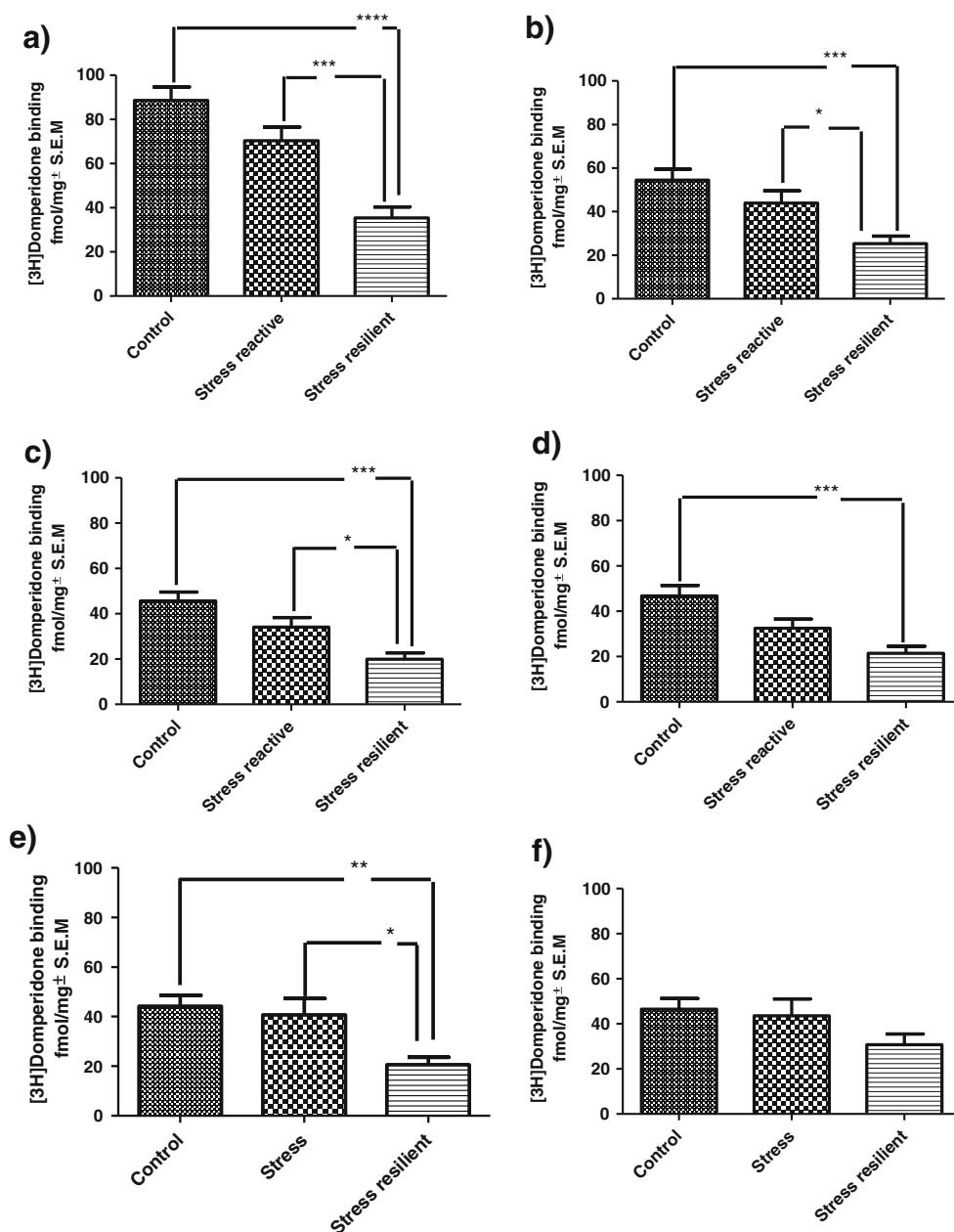

depression. Male rodents are used in this model because males are more biologically stable than females. The dopamine reward circuit in female rats is strongly affected by hormonal changes during the oestrus cycle (Becker 1999). Gonadal hormones, such as oestrogen and progesterone, modulate dopamine activity and receptor levels in the striatum and nucleus accumbens in female rats. In contrast, oestrogen does not affect striatal dopamine release in male rats, and male hormones, such as testosterone, do not affect the brain dopamine reward circuit (Becker 1999; Becker et al. 2005). These factors support the selection of male rats in these experiments.

Behavioural experiments demonstrated that 70 % of rats subjected to the CMS paradigm exhibited a decrease in the consumption of palatable sucrose solution, which suggests

that these rats were vulnerable to stress. The remaining 30 % of the stressed animals were stress resilient, and these animals are often rejected from further analysis, because they do not exhibit the "appropriate" behavioural response to the stress regimen (Bergström et al. 2008).

The stress-resilient group of animals is important for the identification of the molecular factors that underlie the mechanisms of active coping for stress and adversity. The present study demonstrated that stress strongly affected the mesolimbic dopamine circuit in stress-resilient and stress-susceptible animals. This observation is consistent with that of Krishnan et al. (2007) and Blugeot et al. (2011), who demonstrated that stress-resilient and stress-susceptible animals exhibit elevated serum corticosterone levels. The
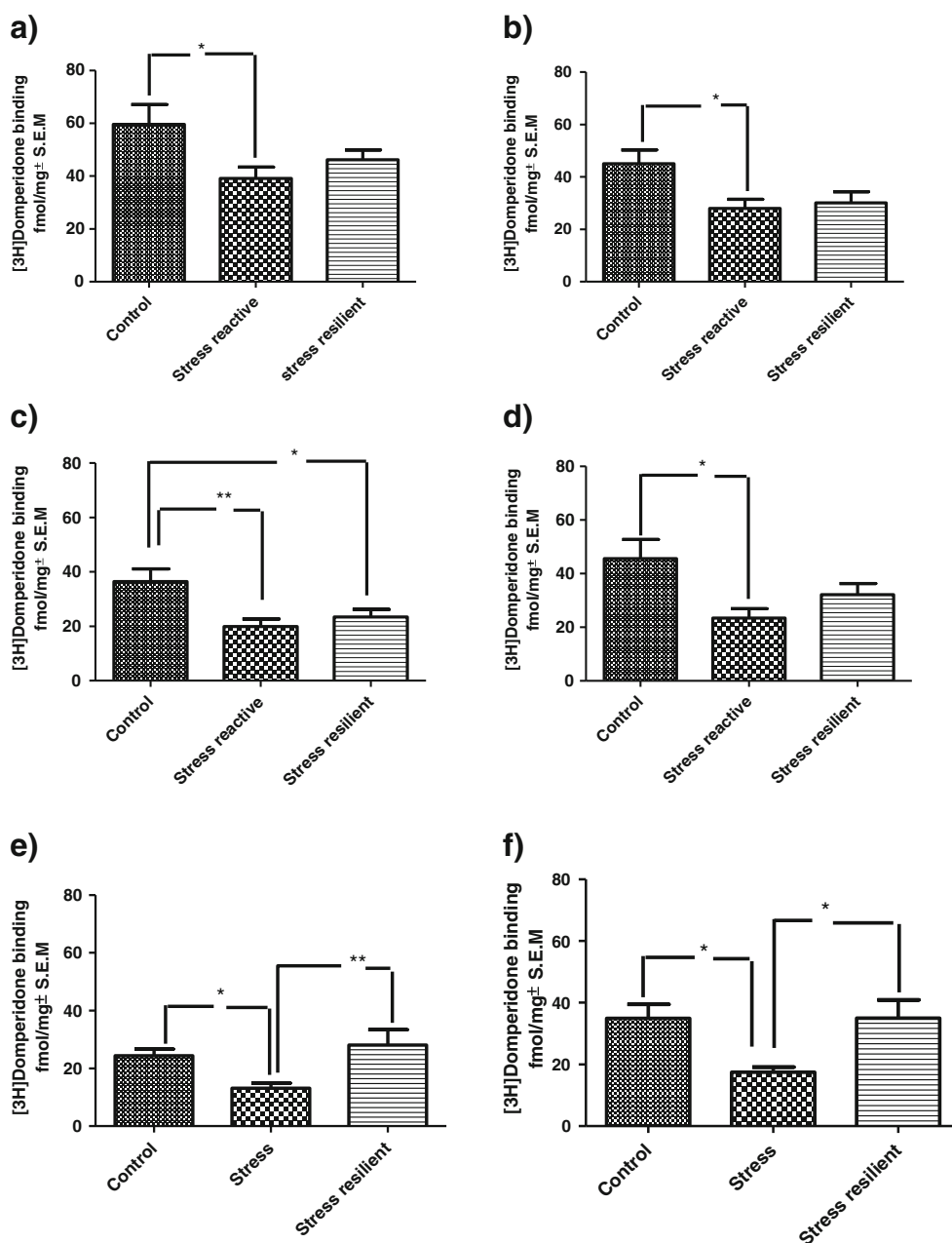
Fig. 4 The effect of 2 weeks of chronic mild stress on dopamine D_2 receptor mRNA levels in various regions of the rat brain. **a** Lateral striatum; **b** medial striatum; **c** nucleus accumbens septi, core; **d** nucleus accumbens septi, shell; **e** lateral ventral tegmental area; **f** medial ventral tegmental area. Data represent the means ± SEM; $n=7$–10; ANOVA, non-significant
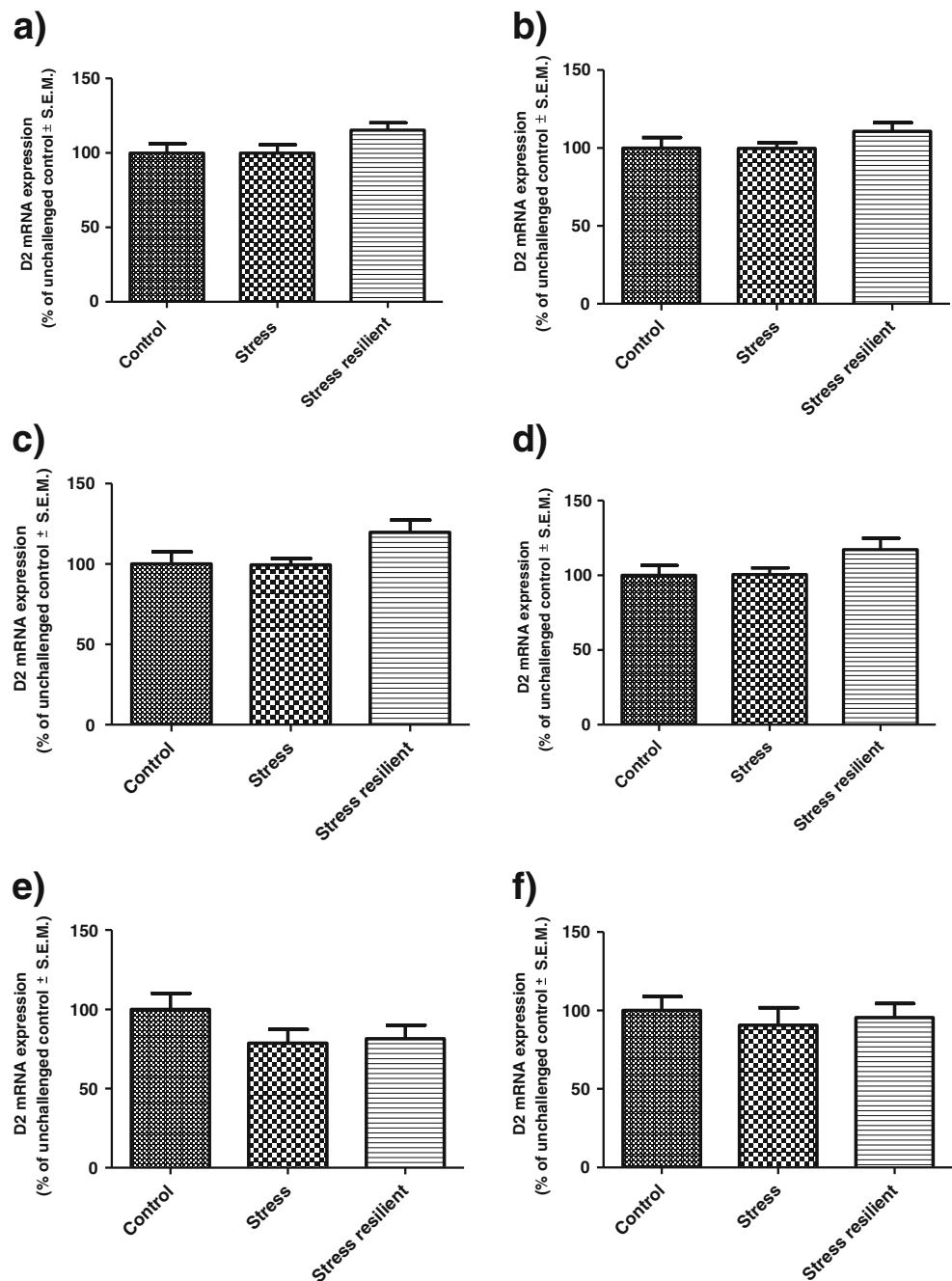
stress-resilient group subjected to CMS exhibited a down-regulation of dopamine D_2 receptors in all of the examined brain structures except the medial VTA. This effect was significant following 2 weeks of the CMS procedure when compared with control animals, which suggests an internal-isation of the receptors in response to the enhanced dopamine release, because no down-regulation of D_2 receptor biosynthesis (i.e. mRNA) was observed. However, the extension of CMS for 3 additional weeks (5 weeks total) produced a different effect, which clearly supports active processes in stress-resilient animals. This group displayed strong neurobiological plasticity in dopamine D_2 receptor

density and mRNA expression, in contrast to stress-reactive animals. These alterations buffered the influence of stress and thus normalised the mesolimbic dopamine circuit via the elevation of dopamine D_2 receptor biosynthesis to control levels in stress-resilient animals. This effect was not observed in stress-reactive rats after 5 weeks of CMS. The normalisation of the brain reward circuit in stress-resilient animals may be the result of elevated dopamine D_2 auto-receptor biosynthesis in the VTA region, which decreases the firing rate of dopamine neurons that project to the ventral striatum in response to stress. D_2 mRNA in the striatum and nucleus accumbens is responsible for the

biosynthesis of dopamine D_2 receptors in non-dopaminergic cells in these brain regions. We observed alterations in D_2 mRNA levels in the VTA and regions receiving projections from dopaminergic cells after 5 weeks of CMS. Therefore, we conclude that the biosynthesis of both pre- and post-synaptic dopaminergic D_2 receptors was increased. These results further support the hypothesis that stress resilience is a biochemically and biologically active and dynamic process that occurs very early at multiple levels in organisms that face trauma or adversity (Haglund et al. 2007; Kanarik et al. 2011). Moreover, stress-reactive animals may exhibit a delayed dopamine D_2 receptor response to stress when compared with stress-resilient animals, which produces an anhedonic mood. However, this hypothesis must be tested in further studies.

Chronic and unpredictable stress regimens decrease dopamine D_2 mRNA expression and receptor density in the mesoaccumbens circuit in stress-reactive animals (Puglisi-Allegra et al. 1991; Papp et al. 1994; Dziedzicka-Wasylewska et al. 1997; Cabib et al. 1998; Zhu et al. 2010). Dopamine D_2 receptor density also increases after chronic antidepressant treatment, which supports the potential antidepressant efficacy of D_2 receptor activation (Gershon et al. 2007; Dunlop and Nemeroff 2007). Therefore, decreased D_2 receptor density in mesolimbic system may be a primary factor in reward subsensitivity and anhedonia, which is a core symptom of depression (Papp et al. 1994; Cabib et al. 1998). However, few studies have focused on the molecular, neurobiological and biochemical changes in dopamine D_2 signalling in stress-

Fig. 6 The effect of 5 weeks of chronic mild stress exposure dopamine D_2 receptor mRNA levels in various regions of the rat brain. **a** Lateral striatum; **b** medial striatum; **c** nucleus accumbens septi, core; **d** nucleus accumbens septi, shell; **e** lateral ventral tegmental area; **f** medial ventral tegmental area. Data represent the means ± SEM; $n=10$–11; ANOVA followed by Bonferroni's multiple comparison post hoc test; $*p<0.05$; $**p<0.01$; $***p<0.001$
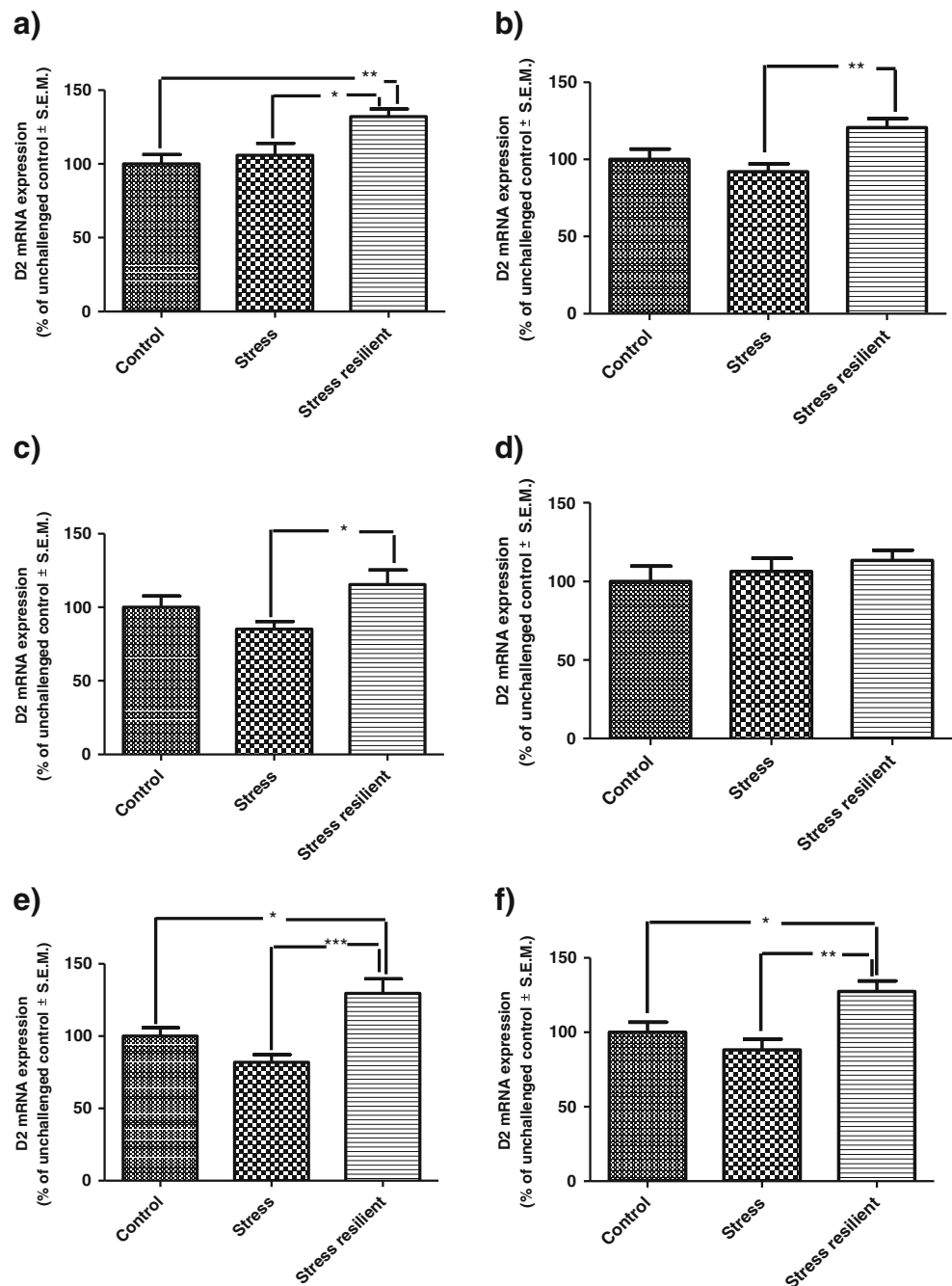
resilient animals (Krishnan et al. 2007; Bergström et al. 2008; Kanarik et al. 2011; Blugeot et al. 2011). Bergström et al. (2008) have demonstrated that restraint stress and 5 weeks of CMS do not alter dopamine D_2 receptor mRNA expression in the nucleus accumbens of control, stress-reactive and stress-resilient rats. However, our work focused on the terminal and brain regions that contain cell bodies (medial and lateral VTA) of the dopamine mesolimbic system. Furthermore, we used a mixture of three oligonucleotides that are complementary to three different regions of the D_2 receptor mRNA to enhance the sensitivity of in situ hybridisation. The

statistically significant parallel increases in dopamine D_2 mRNA expression and receptor density in the medial and lateral VTA in stress-resilient animals demonstrate that the mesolimbic circuit can effectively adapt to new circumstances and allow the animals to cope with adversity despite their sensitivity to chronic stress. In contrast, stress-reactive rats exhibited a significant and persistent decrease in dopamine D_2 receptor density and unchanged mRNA expression levels. This phenomenon suggests that these animals exhibit an impaired mesoaccumbens reward circuit that cannot actively cope with stress (Stamford et al. 1991; Cabib et al. 1998; Kruk et al. 1998; Di Chiara et al. 1999; Cao et al. 2010).

We used domperidone to visualise dopamine D_2 receptor density in various brain regions. This compound is a specific antagonist that recognises the high- and low-affinity conformational states of the D_2 receptor (Seeman et al. 2003, 2005). Therefore, the observed changes in [^{3}H]domperidone receptor binding in the VTA and NAcc in both groups of animals may be the sum of two phenomena: the down-regulation of D_2 dopamine receptors and the switching of their conformation into a low-affinity state (Rogoz and Dziedzicka-Wasylewska 1999; Seeman et al. 2005).

Our study suggests that the links between behavioural and biochemical responses to stress are not straightforward. The behavioural response to stress depends on various factors and systems and their mutual relationships at different levels (Manji et al. 2001). The behavioural CMS experiment suggests that resilient animals exhibit de facto resistance to stress, which is expressed as unaltered consumption of a 1 % sucrose solution. Biochemical analyses indicated an imbalance in mesolimbic dopamine circuit in stress-resilient rats as demonstrated by a decreased in [3H]domperidone binding when compared with stress-reactive group after 2 weeks of CMS. Mesolimbic D_2 receptor density in the stress-resilient group, but not the stress-reactive group, returned to normal levels after 5 weeks of CMS in all examined brain regions, except the NAcc core region, which is related to the caudate and putamen, and, in effect, less associated with the limbic reward system (Deutch and Cameron 1992). Stress influences dopamine D_2 receptors and other catecholamine circuits and these common fluctuations produce specific behavioural responses to stress that may not be directly related to changes in dopamine D_2 receptors alone. An examination of all of these factors is very important for a profound understanding on the mechanisms underlying stress resilience. These results clearly demonstrate that despite earlier blunting, the activation of dopamine receptor biosynthesis in the dopamine mesoaccumbens system in stress-resilient rats is involved in active coping for stressful experiences, while in stress-reactive animals, this phenomenon does not occur or may be delayed (Cabib and Puglisi-Allegra 1994; Puglisi-Allegra et al. 1991). However, further investigation is required to delineate the precise mechanisms underlying the time-dependent normalisation of dopamine D_2 receptor levels in the mesolimbic system.

References

Becker JB (1999) Gender differences in dopamine function in striatum and nucleus accumbens. Pharmacol Biochem Behav 64(4):803–812. doi:10.1016/S0091-3057(99)00168-9

Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, Herman JP, Marts S, Sadee W, Steiner M, Taylor J, Young E (2005) Strategies and methods for research on sex differences in brain and behavior. Endocrinology 146(4):1650–1673. doi:10.1210/en.2004-1142

Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z (2005) Behavioural and neurochemical effects induced by chronic mild stress applied to two different rats strains. Behav Brain Res 161(1):45–59. doi:10.1016/j.bbr.2005.01.005

Bergström A, Jayatissa MN, Mørk A, Wiborg O (2008) Stress sensitivity and resilience in the chronic mild stress rat model of depression; an in situ hybridization study. Brain Res 1196:41–52. doi:10.1016/j.brainres.2007.12.025

Bielajew C, Konkle AT, Merali Z (2002) The effects of chronic mild stress on male Sprague–Dawley and Long Evans rats: I. Biochemical and physiological analyses. Behav Brain Res 136(2):583–592. doi:10.1016/SO166-4328(02)00222-X

Blugeot A, Rivat C, Bouvier E, Molet J, Mouchard A, Zeau B, Bernard C, Benoliel JJ, Becker C (2011) Vulnerability to depression: from brain neuroplasticity to identification of biomarkers. J Neurosci 31(36):12889–12899. doi:10.1523/JNEUROSCI.1309-11.2011

Cabib S, Puglisi-Allegra S (1994) Opposite responses of mesolimbic dopamine system to controllable and uncontrollable aversive experiences. J Neurosci 14(5 Pt 2):3333–3340

Cabib S, Puglisi-Allegra S (1996a) Different effects of repeated stressful experiences on mesocortical and mesolimbic dopamine metabolism. Neuroscience 73(2):375–380. doi:10.1016/0306-4522(96)00750-6

Cabib S, Puglisi-Allegra S (1996b) Stress, depression and the mesolimbic dopamine system. Psychopharmacology (Berl) 128(4):331–342. doi:10.1007/s002130050142

Cabib S, Giardino L, Calzá L, Zanni M, Mele A, Puglisi-Allegra S (1998) Stress promotes major changes in dopamine receptor densities within the mesoaccumbens and nigrostriatal systems. Neuroscience 84(1):193–200. doi:10.1016/S0306-4522(97)00468-5

Cao JL, Covington HE 3rd, Friedman AK, Wilkinson MB, Walsh JJ, Cooper DC, Nestler EJ, Han MH (2010) Mesolimbic dopamine neurons in the brain reward circuit mediate susceptibility to social defeat and antidepressant action. J Neurosci 30(49):16453–16458. doi:10.1523/JNEUROSCI.3177-10.2010

Cicchetti D (2010) Resilience under conditions of extreme stress: a multilevel perspective. World Psychiatry 9(3):145–154

Dailly E, Chenu F, Renard CE, Bourin M (2004) Dopamine, depression and antidepressants. Fundam Clin Pharmacol 18(6):601–607. doi:10.1111/j.1472-8206.2004.00287.x

Deutch AY, Cameron DS (1992) Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience 46(1):49–56. doi:10.1016/0306-4522(92)90007-O

Di Chiara G, Tanda G (1997) Blunting of reactivity of dopamine transmission to palatable food: a biochemical marker of anhedonia in the CMS model? Psychopharmacology (Berl) 134(4):351–353. doi:10.1007/s002130050465, discussion 371–7

Di Chiara G, Loddo P, Tanda G (1999) Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. Biol Psychiatry 46(12):1624–1633. doi:10.1016/S0006-3223(99)00236-X

Dunlop BW, Nemeroff CB (2007) The role of dopamine in the pathophysiology of depression. Arch Gen Psychiatry 64(3):327–337

Dziedzicka-Wasylewska M, Willner P, Papp M (1997) Changes in dopamine receptor mRNA expression following chronic mild stress and chronic antidepressant treatment. Behav Pharmacol 8(6–7):607–618

Feder A, Nestler EJ, Charney DS (2009) Psychobiology and molecular genetics of resilience. Nat Rev Neurosci 10(6):446–457. doi:10.1038/nrn2649

Finlay JM, Zigmond MJ (1997) The effects of stress on central dopaminergic neurons: possible clinical implications. Neurochem Res 22(11):1387–1394. doi:10.1023/A:1022075324164

Gershon AA, Vishne T, Grunhaus L (2007) Dopamine D2-like receptors and the antidepressant response. Biol Psychiatry 61(2):145–153. doi:10.1016/j.biopsych.2006.05.031

Haglund ME, Nestadt PS, Cooper NS, Southwick SM, Charney DS (2007) Psychobiological mechanisms of resilience: relevance to prevention and treatment of stress-related psychopathology. Dev Psychopathol 19(3):889–920. doi:10.1017/S0954579407000430

Imperato A, Cabib S, Puglisi-Allegra S (1993) Repeated stressful experiences differently affect the time-dependent responses of the mesolimbic dopamine system to the stressor. Brain Res 601 (1–2):333–336. doi:10.1016/0006-8993(93)91732-8

Kanarik M, Alttoa A, Matrov D, Kõiv K, Sharp T, Panksepp J, Harro J (2011) Brain responses to chronic social defeat stress: effects on regional oxidative metabolism as a function of a hedonic trait, and gene expression in susceptible and resilient rats. Eur Neuropsychopharmacol 21(1):92–107. doi:10.1016/j.euroneuro.2010.06.015

Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ (2007) Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131(2):391–404. doi:10.1016/j.cell.2007.09.018

Kruk ZL, Cheeta S, Milla J, Muscat R, Williams JE, Willner P (1998) Real time measurement of stimulated dopamine release in the conscious rat using fast cyclic voltammetry: dopamine release is not observed during intracranial self stimulation. J Neurosci Methods 79(1):9–19. doi:10.1016/S0165-0270(97)00156-8

Larisch R, Klimke A, Vosberg H, Löffler S, Gaebel W, Müller-Gärtner HW (1997) In vivo evidence for the involvement of dopamine-D2 receptors in striatum and anterior cingulate gyrus in major depression. NeuroImage 5(4 Pt 1):251–260. doi:10.1006/nimg.1997.0267

Manji HK, Drevets WC, Charney DS (2001) The cellular neurobiology of depression. Nat Med 7(5):541–547. doi:10.1038/87865

Papp M, Klimek V, Willner P (1994) Parallel changes in dopamine D2 receptor binding in limbic forebrain associated with chronic mild stress-induced anhedonia and its reversal by imipramine. Psychopharmacology (Berl) 115(4):441–446. doi:10.1007/BF02245566

Puglisi-Allegra S, Kempf E, Schleef C, Cabib S (1991) Repeated stressful experiences differently affect brain dopamine receptor subtypes. Life Sci 48(13):1263–1268. doi:10.1016/0024-3205(91)90521-C

Rogoz R, Dziedzicka-Wasylewska M (1999) Effects of antidepressant drugs on the dopamine D2/D3 receptors in the rat brain differentiated by agonist and antagonist binding—an autoradiographic analysis. Naunyn Schmiedebergs Arch Pharmacol 359(3):178–186. doi:10.1007/PL00005340

Rowland LM (2011) Who is resilient to depression? Multimodal imaging of the hippocampus in preclinical chronic mild stress model may provide clues. Biol Psychiatry 70(5):406–407. doi:10.1016/j.biopsych.2011.07.002

Seeman P, Tallerico T, Ko F (2003) Dopamine displaces [3H]domperidone from high-affinity sites of the dopamine D2 receptor, but not [3H]raclopride or [3H]spiperone in isotonic medium: implications for human positron emission tomography. Synapse 49(4):209–215. doi:10.1002/syn.10232

Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, Grandy DK, Premont RT, Sotnikova TD, Boksa P, El-Ghundi M, O'dowd BF, George SR, Perreault ML, Männistö PT, Robinson S, Palmiter RD, Tallerico T (2005) Dopamine supersensitivity correlates with D2High states, implying many paths to psychosis. Proc Natl Acad Sci U S A 102(9):3513–3518. doi:10.1073/pnas.0409766102

Stamford JA, Muscat R, O'Connor JJ, Patel J, Trout SJ, Wieczorek WJ, Kruk ZL, Willner P (1991) Voltammetric evidence that subsensitivity to reward following chronic mild stress is associated with increased release of mesolimbic dopamine. Psychopharmacology (Berl) 105(2):275–282. doi:10.1007/BF02244322

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 93(3):358–364. doi:10.1007/BF00187257

Willner P, Hale AS, Argyropoulos S (2005) Dopaminergic mechanism of antidepressant action in depressed patients. J Affect Disord 86 (1):37–45. doi:10.1016/j.jad.2004.12.010

Yadid G, Overstreet DH, Zangen A (2001) Limbic dopaminergic adaptation to a stressful stimulus in a rat model of depression. Brain Res 896(1–2):43–47. doi:10.1016/S0006-8993(00)03248-0

Zhu X, Li T, Peng S, Ma X, Chen X, Zhang X (2010) Maternal deprivation-caused behavioral abnormalities in adult rats relate to a non-methylation-regulated D2 receptor levels in the nucleus accumbens. Behav Brain Res 209(2):281–288. doi:10.1016/j.bbr.2010.02.005

Zhu X, Peng S, Zhang S, Zhang X (2011) Stress-induced depressive behaviors are correlated with Par-4 and DRD2 expression in rat striatum. Behav Brain Res 223(2):329–335. doi:10.1016/j.bbr.2011.04.052