

Isolated Flinders Sensitive Line rats have decreased dopamine D2 receptor mRNA

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Social isolation has profound effects on animal behavior and dopamine systems. We investigated the effect of social isolation on the dopamine receptor and neuropeptide mRNAs in the brain reward system in an animal model of depression, the Flinders Sensitive Line rats and Sprague–Dawley controls. We demonstrate that socially isolated but not group housed Flinders sensitive line rats had lower dopamine D2 receptor mRNA levels compared with Sprague–Dawley rats. Isolated and group housed Flinders Sensitive Line rats

had higher levels of dopamine D1 receptor and substance P and enkephalin but not dynorphin mRNAs when compared with Sprague–Dawley rats. Our findings of decreased dopamine D2 receptor levels in socially isolated Flinders Sensitive Line rats suggest that low D2 receptor expression may play a role in pathophysiology of depression. *NeuroReport* 18:1039–1043 © 2007 Lippincott Williams & Wilkins.

Keywords: brain reward system, depression, dopamine receptors, dynorphin, enkephalin, Flinders Sensitive Line (FSL), neuropeptides, social isolation, substance P

Introduction

The mesolimbic dopamine system is a key structure in motivational behavior and pleasure seeking and it is possible that malfunctioning of the brain reward system is an underlying mechanism of the anhedonia experienced in depressive illness [1]. Moreover, housing conditions can have profound effects on animal behavior and drug response and can induce changes in the dopaminergic system [2,3]. In particular, the family of dopamine D2 receptors can be regulated by environmental stimuli [2,3]. Peer separation or social isolation is stressful and increases anxiety-like behavior in animals [4]. It is recognized that a combination of genetic and environmental factors plays a crucial role in the pathogenesis of affective disorders. Therefore, one purpose of this study was to investigate the effect of social isolation in adulthood in a genetic animal model of depression, the Flinders Sensitive Line rats (FSL) and Sprague–Dawley (SD) controls. A number of symptoms seen in human depression are mimicked by the FSL rats, and some of these include lower body weight, anhedonia in response to chronic mild stress [5], and reduced physical activity [6]. In the Porsolt's swim test they display increased immobility that is reversible by chronic but not acute antidepressive treatments [5,6]. Thus, the FSL represents an appropriate model for studying neurochemical mechanisms involved in depressive illness. We investigated the effects of 7 weeks of social isolation on animal weight and mRNA regulation in the brain reward system. Possible interactions between strain and housing condition were examined.

Materials and methods

Animals and treatment

The experiment was performed in female FSL rats ($n=16$), bred at the Karolinska Institute, and age-matched female SD rats ($n=16$) (Møllegaard Breeding Centre, Denmark). At the start of the experiment, the animals were 29–30 weeks old. Before the experiment all animals were housed under standard housing conditions (4/cage) with access to food and water *ad libitum*. The animals were randomly assigned two groups, one with standard group housing conditions (4/cage) and one housed in individual cages. These housing conditions were maintained for 7 weeks. The animals were only handled when weighed once a week. All animals had access to food and water *ad libitum* during the whole experiment, and were subjected to a controlled 12-h light:12-h dark schedule (lights on at 07.00 h). After 7 experimental weeks the animals were killed. The brains were rapidly removed and stored at -80°C for analyses of mRNAs encoding dopamine receptors, endogenous opioids and neuropeptides. All animal experiments were approved by the local Ethical Committee for Animal Research in Stockholm.

In-situ hybridization

Coronal brain sections (14 μm) were cut on a cryostat at -20°C , and sections were thawed onto glass slides. Single-stranded oligonucleotide 48-mer DNA probes specific for dopamine D1 receptor mRNA (72–121) [7], dopamine D2 receptor mRNA (772–816) [8], dynorphin (296–345) [9],

enkephalin (235–282) [10], and substance P (20–67) [11] were used. The probes were 3'-end labeled with α - ^{33}P -dATP (Dupont NEN, Wilmington, Delaware, USA) to a specific activity of approximately 1×10^9 cpm/mg using terminal deoxynucleotidyl transferase (Gibco, Täby, Sweden) and hybridized according to a standardized protocol [6]. Autoradiogram films were scanned and optical density values quantified using appropriate software (ImageJ, National Institutes of Health, Maryland, USA). A ^{14}C step standard (Amersham, Buckinghamshire, UK) was included to calibrate optical density readings and convert measured values to nCi/g.

Statistics

To analyze animals' weight, three-way ANOVA (strain \times housing \times time) with repeated measures within and planned comparison post hoc tests was performed. Two-way ANOVAs (strain \times housing) with planned comparison and Scheffé post-hoc tests were used to analyze the different mRNAs within each region in FSL and SD rats that were group housed or socially isolated.

Results

Body weight in group housed and socially isolated Flinders Sensitive Line rats and Sprague–Dawley rats

An overall three-way ANOVA with repeated measures revealed differences between strains ($P < 0.001$), weight gain during the experiment ($P < 0.001$), and an interaction effect between strain and housing in control of weight gain during the experiment ($P < 0.05$). An expected weight difference existed between age-matched SD and FSL rats, independent

of housing condition ($P < 0.001$), SD rats weighing on average 40% more than FSL (374 ± 3.9 and 261 ± 4.6 g) at the start of the experiment. In the SD strain, there was a nonstatistically significant trend towards an increase in weight during the experiment in group housed animals ($P = 0.06$). Socially isolated SD rats increased their weight by 3.1% ($P < 0.001$). In contrast, in the FSL strain group housed animals increased their weight by 3.5% during the course of the experiment ($P < 0.05$), whereas socially isolated FSL rats did not gain any weight during the experiment.

Dopamine receptor and neuropeptide mRNA levels in striatal subregions in group housed and socially isolated Flinders Sensitive Line rats and Sprague–Dawley rats

Levels of mRNAs encoding the dopamine D1 and D2 receptors and the neuropeptides dynorphin, enkephalin, and substance P, were analyzed in striatal subregions including nucleus accumbens.

Analyses of mRNAs encoding the dopamine D2 receptors revealed differences between the two strains. The difference however, was restricted to socially isolated rats. Socially isolated SD rats had higher levels of dopamine D2 receptor mRNAs than socially isolated FSL rats in all striatal subregions including accumbens ($P < 0.05$ – 0.01) (Figs 1 and 2). A trend towards strain \times housing interaction in the lateral caudate putamen ($P = 0.09$) was found.

The FSL strain had higher levels of dopamine D1 receptor mRNA than the SD strain in all analyzed regions in both group housed and socially isolated animals ($P < 0.01$ – 0.001) (Table 1). Housing had no effect on dopamine D1 receptor mRNA levels in either strain.

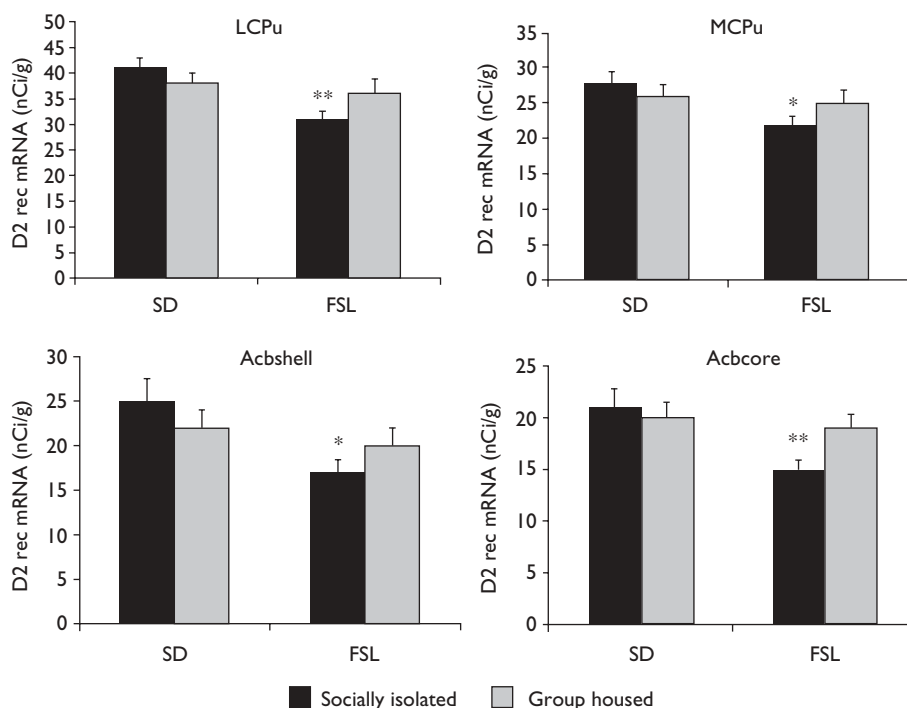


Fig. 1 Dopamine D2 mRNA levels after group housing and social isolation. Quantitative *in situ* hybridization of autoradiograms from Flinders sensitive line rats (FSL) and Sprague–Dawley (SD) rats housed in a group or in social isolation for 7 weeks (all groups $n = 8$). Values are means \pm SEM. * $P < 0.05$, ** $P < 0.01$ indicate significantly lower levels of the dopamine D2 receptor mRNA in socially isolated FSL rats compared with the SD rats. Analyses were performed approximately at the level of Bregma 1.60 mm. LCPu, lateral caudate putamen; MCPu, medial caudate putamen; Acbshell, nucleus accumbens shell; Acbcore, nucleus accumbens core.

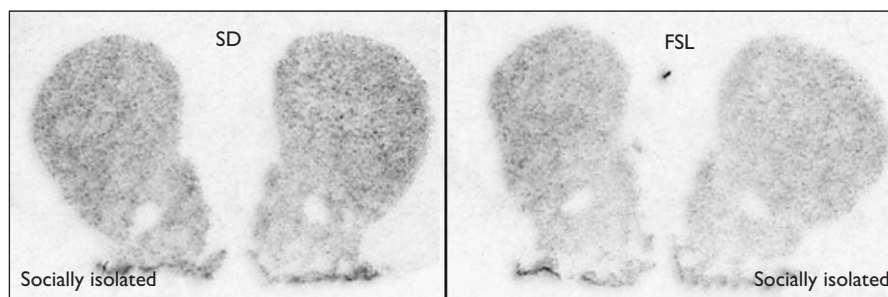


Fig. 2 *In situ* hybridization of dopamine D2 receptor mRNA expression in ventral and dorsal striatum in socially isolated Flinders sensitive line rats (FSL) and Sprague-Dawley (SD) rats. The autoradiogram illustrates dopamine D2 receptor mRNA in SD and FSL rats that have been socially isolated for 7 weeks. Note the decreased expression of dopamine D2 receptor mRNA in lateral and medial caudate putamen, accumbens shell and core in FSL rats compared with the SD rats.

Table 1 Dopamine D1 receptor, dynorphin, enkephalin and substance P mRNA expression. Levels of dopamine D1 receptor, dynorphin, enkephalin and substance P mRNA expression in ventral and dorsal striatum (represented as optical densities) were quantified and are shown as mean nCi/g \pm SEM

| | SD | | FSL | |
|-------------|----------------|-------------------|-------------------|-------------------|
| | Group housed | Socially isolated | Group housed | Socially isolated |
| DA D1 rec | | | | |
| ICPu | 49 \pm 2.0 | 48 \pm 2.2 | 57 \pm 2.1## | 59 \pm 2.2*** |
| mCPu | 47 \pm 2.0 | 46 \pm 1.6 | 54 \pm 1.7## | 56 \pm 1.2*** |
| Acbsshell | 43 \pm 3.8 | 47 \pm 1.9 | 53 \pm 2.5## | 57 \pm 2.0* |
| Acbc core | 34 \pm 2.6 | 34 \pm 2.4 | 43 \pm 1.5## | 44 \pm 1.5** |
| Dynorphin | | | | |
| ICPu | 58 \pm 2.0 | 52 \pm 3.9 | 60 \pm 3.2 | 58 \pm 1.9 |
| mCPu | 65 \pm 3.6 | 58 \pm 1.9 | 63 \pm 2.2 | 57 \pm 1.6 |
| Acbsshell | 130 \pm 6.6 | 131 \pm 9.8 | 134 \pm 5.9 | 133 \pm 4.9 |
| Acbc core | 120 \pm 7.9 | 106 \pm 13.8 | 101 \pm 6.3 | 97 \pm 4.7 |
| Enkephalin | | | | |
| ICPu | 418 \pm 14.0 | 405 \pm 11.6 | 431 \pm 8.7 | 422 \pm 7.4 |
| mCPu | 380 \pm 10.2 | 358 \pm 13.1 | 418 \pm 4.7## | 404 \pm 8.0** |
| Acbsshell | 195 \pm 13.2 | 206 \pm 14.6 | 277 \pm 8.8### | 283 \pm 12.2*** |
| Acbc core | 302 \pm 15.2 | 287 \pm 23.4 | 355 \pm 9.3# | 342 \pm 10.3* |
| Substance P | | | | |
| ICPu | 121 \pm 7.5 | 123 \pm 7.5 | 171 \pm 13.7### | 167 \pm 6.7*** |
| mCPu | 83 \pm 4.9 | 78 \pm 3.8 | 117 \pm 9.1### | 113 \pm 7.2*** |
| Acbsshell | 133 \pm 9.0 | 132 \pm 9.8 | 155 \pm 5.8 | 158 \pm 6.1 |
| Acbc core | 55 \pm 4.3 | 52 \pm 4.6 | 72 \pm 2.0# | 74 \pm 6.8** |

Acbc core, nucleus accumbens core; Acbsshell, nucleus accumbens shell; FSL, Flinders sensitive line rats; LCPu, lateral caudate putamen; MCPu, medial caudate putamen; SD, Sprague-Dawley rats.

SD and FSL rats randomized into two different housing conditions, half housed in standard conditions 4 rats/cage (group housed), and half housed in single cages (socially isolated). These housing conditions were maintained for 7 weeks.

Strain difference between group housed animals. # P < 0.05, ## P < 0.01, ### P < 0.001.

**** Strain difference between socially isolated animals. * P < 0.05, ** P < 0.01, *** P < 0.001.

Dynorphin mRNA levels did not differ between the SD and the FSL strain. Social isolation decreased dynorphin mRNA in the medial caudate putamen levels when data from SD and FSL were pooled (P < 0.05) (data not shown). There was also a trend towards a decrease in this region in socially isolated SDs compared with group housed SDs (P = 0.08) (Table 1).

Enkephalin mRNA levels were higher in both group housed and socially isolated FSL compared with SD in medial caudate putamen, accumbens shell and core

(P < 0.05–0.001) (Table 1). Housing did not affect enkephalin mRNA levels within the strains. Pooling SD and FSL data, however, indicated a trend to a decrease in enkephalin mRNA in medial caudate putamen (P = 0.07) (data not shown).

Substance P mRNA levels differed between the strains, FSL having higher levels than SD rats in lateral and medial caudate putamen (P < 0.001), and accumbens core (P < 0.05–0.01), in both group housed and socially isolated animals. FSL rats also had higher levels in accumbens shell in socially isolated animals (P < 0.01) and a strong trend towards increased levels in group housed animals (P = 0.06) (Table 1) compared with SD. No housing or interaction effects were found.

Discussion

Stress, genetic vulnerability and dopaminergic neurotransmission have been implicated in the pathogenesis of mental illness, including depressive illness [12,13]. A line of evidence suggests a role for the D2 receptor in depression [12,13], and that the social environment can influence the expression of dopamine D2 receptors [2,3].

In this study we compared dopamine D1 and D2 receptor mRNA levels in brain reward pathways in FSL rats, a genetic animal model of depression, to SD control rats. Moreover, we analyzed levels of mRNAs encoding the neuropeptides substance P, enkephalin and dynorphin, all of which are connected to dopamine transmission. No differences in the D2 receptor mRNA levels between group housed FSL and SD rats were found. In contrast, socially isolated FSL rats had lower levels of D2 receptor mRNA in medial and lateral caudate-putamen and accumbens core and shell compared with SD controls.

Environmental influence on D2 receptor has previously been documented in a PET imaging study that showed no difference in availability of dopamine D2 receptor in basal ganglia in individually housed male cynomolgus macaques. Upon social housing the availability of dopamine D2 receptors in dominant monkeys increased whereas no change was detected in subordinate monkeys [2]. Recently, it was demonstrated that low levels of D2 receptor availability were independent of dopamine release, and thus possibly can be explained by decreased dopamine D2 receptor density [14]. PET analysis does not distinguish between presynaptic and postsynaptic dopamine receptors. The resolution of PET is limited and the analyzed region is defined as basal ganglia, which includes both accumbens

and caudate putamen. In this study the dopamine D2 receptor mRNA levels were analyzed in rats in medial and lateral caudate putamen as well as accumbens core and shell using in-situ hybridization. Most mRNAs accumulate in the cell body and not in nerve terminals; thus, the difference in dopamine D2 receptor mRNA levels in isolated FSL and SD rats is only caused by differences in postsynaptic dopamine D2 receptor mRNAs.

Data from rodent models suggest that chronic stress results in decreased dopamine release in the ventral striatum [15], followed by a decreased sensitivity to dopaminergic D2-agonists and a decrease in D2 receptor levels in the nucleus accumbens [16]. The different patterns of D2 receptor mRNA regulation in FSL and SD rats suggest that a strain-specific trait may regulate adaptation of the striatal dopaminergic pathways to the chronic mild stress of social isolation. Some characteristics of the FSL strain support the suggestion that this strain is sensitive to mild stress. After the application of chronic mild stress the FSL strain, which does not normally express anhedonia symptoms, exhibited a greater decrease in saccharin intake than the control strain [17], suggesting that the FSL rat is prone to stress-induced anhedonia. Furthermore, stress-induced anhedonia results from changes in the D2 receptor function in the nucleus accumbens [16]. Thus, these results are in line with a strain-specific response to social isolation that involves regulation of dopamine D2 receptor levels in FSL rats.

Human genetics indicate that the DRD2 TaqI A1 allele of the dopamine D2 receptor gene is implicated in anxiety, depression, posttraumatic stress disorder [13] and associated with reduced dopamine D2 receptor binding [18,19]. Thus, taken together animal studies including FSL rats and human in-vivo PET studies, *post mortem* analysis and genetics are in line with hypothesis that altered dopamine D2 receptor binding, mRNA levels and dopamine D2 gene mutation could have an important role in affective disorders.

In this study social isolation induced strain differences in dopamine D2 receptor mRNA in both ventral and dorsal striatum. The mesolimbic dopamine projection, which is essential for motivation and reward, terminates in accumbens whereas the nigrostriatal pathway, which is more involved in motor control, terminates in caudate putamen. An anatomical dichotomy exists between the limbic medial caudate putamen and nucleus accumbens shell which are more important for mood and reward than the accumbens core and dorsal caudate putamen [20]. Psychomotor retardation is a characteristic of depressed individuals. Similarly, FSL rats are less active in the open-field test and show decreased activity in running-wheels [6]. Thus, the FSL strain displays features of anhedonia, and decreased motivation and locomotor activity. Even though the medial and lateral division of caudate putamen and nucleus accumbens core and shell to a great extent functionally interact, the subdivisions might have a different impact on expression of specific traits such as motor behavior or anhedonia-like behavior. The lower expression of dopamine D2 receptor mRNA in the accumbens shell and medial caudate putamen after social isolation could possibly explain the stress-induced anhedonia, whereas the lower expression in accumbens core and dorsal striatum could be involved in the lower motor activity observed in this strain.

Although social isolation had a strain specific effect on D2 receptor mRNA regulations the dopamine D1 receptor and dynorphin, enkephalin and substance P mRNAs were unaffected within the strains. The FSL strain had a higher expression of the dopamine D1 receptor, and the neuropeptides enkephalin and substance P mRNAs compared to the SD strain that were independent of housing condition. Interestingly, the findings of elevated substance P are in line with increased concentrations found in cerebrospinal fluid of depressed patients [21] and the attempts to treat depression by blocking the tachykinin NK1 receptor [22]. Furthermore, the FSL rats have decreased extracellular dopamine levels compared with the SD strains [23]. Previously, it has been demonstrated that low extracellular dopamine levels in the striatum increase enkephalin mRNA in the caudate putamen [24]. This is consistent with our findings of elevated levels of enkephalin in this strain.

Decreased appetite and reduced body weight are features frequently observed in depressed patients (DSM IV). In this study, there was a substantial weight difference between age-matched FSL and SD rats, the latter weighing about 40% more at the start of the experiment. The findings of lower body weight in FSL are in accordance with previous reports [5] and with the weight reduction seen in the maternal separation model of depression [25]. Seven weeks of social isolation had a differential effect on weight of FSL and SD rats. Although socially isolated SD rats increased their body weight during the course of the experiment, even more than group housed SD did, socially isolated FSL did not. In contrast, group housed FSL rats increased their weight by 3.5%, again suggesting that the depressive-like phenotype of the FSL strain may be accentuated by the mild stress of social isolation.

Conclusion

We present data of a postsynaptic dopamine D2 receptor response to social isolation in a genetic animal model of depression. Together with previous data from human postmortem studies on dopamine D2 receptor analysis and mutations in the dopamine D2 receptor gene in patients suffering from affective disorders our data support the notion that dysfunction of the D2 receptor may play a role in the pathophysiology of affective disorders.

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