

Housing conditions modulate escitalopram effects on antidepressive-like behaviour and brain neurochemistry



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Abstract

Despite limited understanding of the pathophysiology of depression and the underlying mechanisms mediating antidepressant effects, there are several efficient treatments. The anhedonia symptoms of depression are characterized by decreased motivation and drive and imply possible malfunctioning of the mesolimbic dopamine system, whereas cognitive deficits might reflect decreased plasticity in hippocampus. In female Flinders Sensitive Line (FSL) rats, a model of depression, we compared the effects of three long-term antidepressant treatments: voluntary running, escitalopram and the combination of both on antidepressant-like behaviour in the Porsolt swim test (PST), and on regulation of mRNA for dopamine and neuropeptides in striatal dopamine pathways and brain-derived neurotrophic factor (BDNF) in hippocampus. Escitalopram diet attenuated running behaviour in FSL rats but not in non-depressed controls rats. In the PST the running group had increased climbing activity (noradrenergic/dopaminergic response), whereas the combination of escitalopram and running-wheel access increased swimming (serotonergic response). Running elevated mRNA for dynorphin in caudate putamen and BDNF in hippocampus. The combined treatment down-regulated D₁ receptor and enkephalin mRNA in accumbens. Escitalopram alone did not affect behaviour or mRNA levels. We demonstrate a novel behavioural effect of escitalopram, i.e. attenuation of running in 'depressed' rats. The antidepressant-like effect of escitalopram was dependent on the presence of a running wheel, but not actual running indicating that the environment influenced the antidepressant effect of escitalopram. Different patterns of mRNA changes in hippocampus and brain reward pathways and responses in the PST by running and escitalopram suggest that antidepressant-like responses by running and escitalopram are achieved by different mechanisms.

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Introduction

The wide variety of symptoms in depressive illness indicates that several brain regions are implicated in the underlying pathophysiology. Anhedonia, the inability to experience pleasure, is one of the core symptoms of major depression (melancholia). Hypothetically, a malfunctioning brain reward system could

account for this impairment. The nucleus accumbens (NAc) is the site of the dopaminergic cell terminals originating in the ventral tegmental area (VTA) (Björklund and Lindvall, 1984; Ungerstedt, 1971) and this pathway plays a critical role in mediating brain reward (Di Chiara and Imperato, 1988). Other symptoms in depressed patients are cognitive impairments. Brain-derived neurotrophic factor (BDNF) is important for brain plasticity as it was shown to have an important role for long-term potentiation, learning and memory (Poo, 2001). While the majority of patients show improvement after treatment with selective serotonin reuptake inhibitors (SSRIs), the mechanisms in brain reward pathways, which could be important

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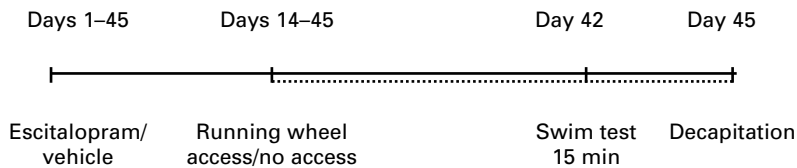


Figure 1. A schematic illustration of the experimental design. On day 1, the animals were administered either escitalopram ($n=24$) or vehicle diet ($n=24$). From day 14 to the end of the experiment (day 45), half of the rats were housed in cages containing a running wheel ($n=32$). On day 42, a modified Porsolt swim test was performed on all FSL animals ($n=32$). All animals were sacrificed on day 45.

for their efficacy, are insufficiently understood (Angelucci et al., 2005; Nestler et al., 2002; Wong and Licinio, 2001). In general, it takes 3–4 wk of treatment before there is a significant difference between responses to an antidepressant drug vs. a placebo (Wong and Licinio, 2001). The delayed onset of SSRIs suggests that adaptive mechanisms rather than enhanced serotonergic neurotransmission per se are responsible for the alleviation of depressive symptoms.

Physical activity also has beneficial effects in the treatment of depression (Babyak et al., 2000; Martinsen et al., 1985). Consistent with these findings, we recently demonstrated that running is an effective antidepressant in a rat model of depression, the Flinders Sensitive Line (FSL). Access to a running wheel during 30 d decreased immobility in the Porsolt swim test (PST) in the FSL strain (Bjørnebekk et al., 2005, 2006). Different types of antidepressants have differential effect on the behaviour in the PST. Whereas the SSRI-type drugs enhance swimming behaviour, drugs that act on norepinephrine transmission increase climbing behaviour (Cryan et al., 2005; Detke et al., 1995; Reneric and Lucki, 1998). To our knowledge there are no reports on whether the antidepressant-like effect of running is associated with a serotonergic or a noradrenergic behavioural response in the PST.

The FSL rats exhibit some characteristics that resemble anhedonia symptoms of depression such as less activity in open field (Overstreet and Russell, 1982), reduced voluntary running (Bjørnebekk et al., 2005), lower rate of bar pressing for water and food reward (Overstreet and Russell, 1982), and increased anhedonia in response to mild stress (Pucilowski et al., 1993). Moreover, microdialysis studies have demonstrated disturbed serotonergic and dopaminergic neurotransmission in striatum and NAc of FSL rats (Dremencov et al., 2005; Zangen et al., 2001).

In view of the anhedonia symptoms in depressed patients and FSL rats we aimed to explore biochemical adaptations in the brain reward system in FSL

rats after different antidepressant treatments. We compared the effect of running, escitalopram, and a combination of these treatments on antidepressant-like parameters in the PST, and on regulation of mRNA for dopamine (DA) receptors and neuropeptides that are regulated by DA in the brain reward system. Moreover we investigated BDNF mRNA regulation in hippocampus after the different antidepressant treatments.

Female rats were used since the prevalence of depressive disorders is higher in women (about two-thirds of depressed patients are women) and fewer both preclinical and clinical studies have been conducted on females.

Method

Animals

Female FSL rats ($n=32$) bred at the Karolinska Institute and age-matched female Sprague–Dawley rats (SD, $n=16$) (Møllegaard Breeding Centre Ltd, Denmark) were used. They were aged 22 wk at the beginning of the experiment. Rats were individually housed throughout the experiment in cages ($43 \times 22 \times 20$ cm) with (FSL, $n=16$; SD, $n=16$) or without (FSL, $n=16$) running-wheel access. During the first 13 d of the experiment all rats had identical cages. On day 14, running wheels were placed in half of the cages (Figure 1). The animals were randomly assigned to one of four groups of eight rats each: (1) vehicle diet and no running wheel in the cage (Veh+nRW), (2) escitalopram diet and no running wheel in the cage (Esc+nRW), (3) vehicle diet and running wheel in the cage (Veh+RW), (4) escitalopram diet and running wheel in the cage (Esc+RW). In view of the observed escitalopram–running interaction in this experiment, a focused replication experiment using identical procedures was carried out on 16 FSL rats (eight Veh+RW and eight Esc+RW). Rats had access to food and water ad libitum and were subjected to a controlled 12 h light/dark schedule (lights on 07:00

hours). The Ethical Committee for Animal Research in Stockholm approved the experiments.

Administration of escitalopram

The rats were administered escitalopram or vehicle in pellets 14 d prior to receiving running-wheel access, and throughout the whole experiment according to a method developed by H. Lundbeck A/S (Copenhagen, Denmark) and tested in collaboration with A. Mørk (El Khoury et al., 2006) (Figure 1). The experiment was run in two consecutive steps, each with 16 rats, which includes the two groups on escitalopram (Esc + nRW, Esc + RW). The escitalopram doses were 0.35 g/kg and 0.57 g/kg pellet in the first and second round, respectively, resembling the clinical situation where a range of escitalopram (or other antidepressants) is used to treat depressed patients. Pellet intake was measured every third day and the average consumption of escitalopram per day was calculated. After decapitation, trunk-blood samples were collected and serum levels of escitalopram were measured.

Wheel running

Half of the FSL and all SD rats had access to running wheels (diameter 34 cm; one revolution corresponding to 1.07 m) (Werme et al., 1999, 2000) from experimental day 14 until the end of the experiment (day 45) (Figure 1). Running data were sampled 48 times/d using a computer-based data system with customized software.

PST

A modified swim test was performed as previously described (Bjørnebekk et al., 2005) on day 42 (Figure 1). Transparent cylinders (24 cm diameter) containing 25-cm deep water (25 °C) were used. After 15 min, the animals were removed from the water, dried with towels and placed into a warmed enclosure before being returned to the home cage. The test was video-recorded and later analysed by an observer blind to the treatment condition. Climbing was defined as time spent with upward-directed movements of the forepaws along the side of the swim chamber and swimming was defined as time with horizontal movement in the cylinder.

The animals were sacrificed on day 45, 3 d after the swim test (Figure 1). Trunk blood was collected immediately after decapitation and centrifuged at 3000 g for 10 min and the supernatant stored at -20 °C. The brains were rapidly removed and stored at -80 °C for subsequent histological analysis.

In-situ hybridization

Coronal brain sections (14 µm) were cut on a cryostat at -20 °C, and sections were thawed onto glass slides. The hybridization cocktail contained 50% formamide, 4 × SSC [1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate (pH 7.0)], 1 × Denhardt's solution, 1% Sarcosyl, 0.02 M Na₃PO₄ (pH 7.0), 10% dextran sulfate, 0.06 M dithiothreitol and 0.1 mg/ml sheared salmon sperm DNA. Single-stranded oligonucleotide 48-mer DNA probes specific for DA D₁ receptor mRNA (nt 72–121) (Wilkie et al., 1993), DA D₂ receptor mRNA (nt 772–816) (Guiramand et al., 1995; Pritchett et al., 1988), prodynorphin (nt 296–345) (Douglass et al., 1989), proenkephalin (nt 235–282) (Zurawski et al., 1986), neurotrophin Y (nt 1671–1714) (Larhammar et al., 1987), and preprotachykinin A (PPT-A) (nt 20–67) (Chiwakata et al., 1991) mRNA, which anneals to both substance P and neurokinin A and BDNF (250–298) (Leibrock et al., 1989) were used. The probes were 3'-end labelled with α-³²P[dATP] (DuPont, NEN, Wilmington, DE, USA) to a specific activity of ~1 × 10⁹ cpm/mg using terminal deoxynucleotidyl transferase (Gibco, Täby, Sweden). Hybridization was performed for 18 h in a humidified chamber at 42 °C. Following hybridization, the sections were rinsed 4 × 20 min each in 1 × SSC at 60 °C. Finally, the sections were rinsed in autoclaved water for 10 s, dehydrated in alcohol and air-dried. Thereafter, the slides were exposed to film (Kodak Biomax MR film, Kodak, Rochester, NY, USA) for 5–29 d before being developed. Films were scanned and optical density values quantified in dorsal and ventral striatal subregions using appropriate software (NIH image analysis program, version 1.62). A ¹⁴C step standard (Amersham, Buckinghamshire, UK) was included to calibrate optical density readings and convert measured values into nCi/g.

Statistical analyses

Values are expressed as means ± standard error of the means (S.E.M.). To analyse weight and the effect of treatment on weight, a two-tailed Student's *t* test was applied; unpaired *t* tests to investigate between-group differences and paired *t* tests when repeated measures were calculated. General MANOVAs with planned comparisons were performed to analyse differences in swim, climbing, and running behaviour between the different treatment groups. One-way ANOVA combined with Tukey–Kramer multiple comparisons post-hoc test was used when comparing mRNA levels between the different treatment groups for each specific mRNA in each region.

Table 1. Weight, escitalopram intake and escitalopram serum level

Strain	Weight (g)		Treatment	Increase (%)	Escitalopram	
	Start	End			mg/kg per d	serum ng/ml
FSL	257 ± 3.6	266 ± 4.0	Veh + nRW	1	–	<1
			Esc + nRW	6*	28.1 ± 2.4	24.4 ± 3.7
			Veh + RW	5	–	<1
			Esc + RW	3*	26.9 ± 2.5	29.0 ± 9.7
SD	331 ± 2.9	354 ± 4.0	Veh + RW	9***	–	<1
			Esc + RW	4	26.8 ± 2.3	36.1 ± 6.6

FSL, Flinders Sensitive Line Rats; SD, Sprague–Dawley; Veh + nRW, vehicle + no running wheel; Esc + nRW, escitalopram + no running wheel; Veh + RW, vehicle + running wheel; Esc + RW, escitalopram + running wheel.

Animal weight was measured weekly during the experiment; average strain weights at the beginning and at the end of the experiment are shown as means ± s.e.m. The weight increase seen in the different treatment groups is expressed as percent of increase from the experimental day 1 to the last day of the experiment. In the FSL strain significant increases in weight were found solely in groups receiving escitalopram diet. In contrast, in the SD strain the group on vehicle diet increased weight whereas the group on escitalopram diet did not. Average daily escitalopram intake is shown as mg/escitalopram consumed per kg ± s.e.m. and escitalopram serum level is expressed as means ± s.e.m. There were no differences between the two strains or between treatment groups regarding escitalopram intake or escitalopram serum level increase in weight within a treatment group during the experiment. * $p < 0.05$, *** $p < 0.001$.

Results

Body weight, food intake and escitalopram serum levels

FSL rats ($n = 32$) weighed significantly less than SD rats ($n = 16$) on the first and the last day of the experiment ($p < 0.001$). At the end of the experiment (45 d), strain-specific differences in weight and weight increase between treatment groups were found. In the FSL strain both groups on the escitalopram diet gained weight during the course of the experiment (Esc + RW 3%, Esc + nRW 6%; $p < 0.05$). In contrast, the Veh + nRW did not gain any weight, while the Veh + RW group had a 5% increase in weight ($p = 0.07$). In the SD strain the Esc + RW group did not gain weight during the experiment, whereas the Veh + RW did increase their weight by 9% (Table 1). The calculated average daily dose of escitalopram in FSL and SD rats was 27.5 mg Esc/kg and 26.6 mg Esc/kg, respectively. On the day of sacrifice, after 45 d of treatment, the mean escitalopram serum level was 31.0 ± 4.7 ng/ml with no statistically significant differences between the two strains (Table 1).

Running behaviour

Statistical evaluation of behavioural responses of the two groups (0.35 g and 0.57 Esc/kg pellet respectively) showed no differences and consequently they were merged into one group for further analyses, which is

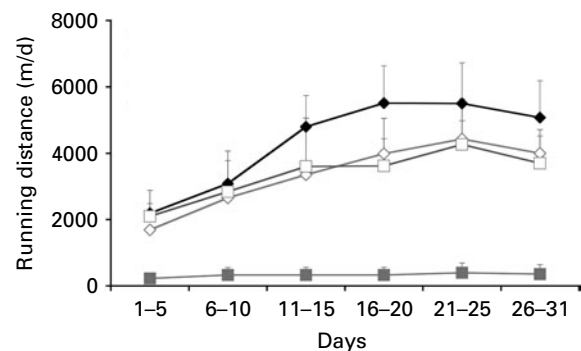


Figure 2. Running behaviour in Flinders Sensitive Line (FSL) and Sprague–Dawley (SD) rats on escitalopram (Esc) and vehicle (Veh) diet. FSL ($n = 16$) and SD ($n = 16$) rats were individually housed with running-wheel access for 30 days. Values are mean ± s.e.m. ($n = 8$ per group). Note the inhibitory effect of escitalopram on running behaviour in FSL but not in SD rats. –◇–, SD Veh; –◆–, SD Esc; –□–, FSL Veh; –■–, FSL Esc.

in line with treatment of depressed patients with different doses of escitalopram. During the first 5 d with running-wheel access, i.e. from days 14 to 18 on escitalopram or vehicle diet, FSL and SD rats on vehicle diet averaged ~2000 m/d (Figure 2), and successively increased their amount of running, before stabilizing around day 20 on 4000–5500 m/d. Escitalopram diet had a profound influence on running behaviour in FSL

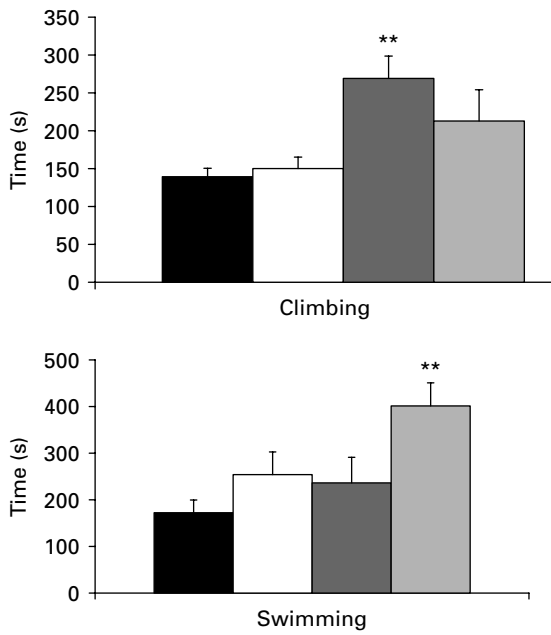


Figure 3. Effect of escitalopram, free wheel-running, and a combination of escitalopram and running on behaviour in the Porsolt swim test. The bar graphs illustrate the swimming and climbing time of FSL rats on vehicle diet in cages with no running wheel (Veh + nRW; ■) or with running-wheel access (Veh + RW; □), on escitalopram diet with no running-wheel (Esc + nRW; □), or with running-wheel access (Esc + RW; ■) ($n=8$ per group). The rats were placed in cylinders filled with water (25 °C) for 15 min. The test was video-recorded and analysed by an observer blind to the experimental conditions. Swimming and climbing times are presented as means \pm S.E.M. (** $p < 0.01$).

($p < 0.001$) but not in SD rats. FSL rats on escitalopram diet ran less than the other three groups ($p < 0.001$). During the first 5-d period, FSL rats on escitalopram reached an average of ~ 200 m/d. Of the eight FSL rats on the escitalopram diet, six of them barely ran at all. They ran on average 30 m/d during the experiment. Two rats ran more; one ~ 500 m/d, and the other averaged 1750 m/d during its best 5-d running period. To confirm the effect of escitalopram on running in FSL rats, we repeated this part of the experiment by giving escitalopram (0.41 g/kg pellet) or vehicle diet 14 d prior to running-wheel access, followed by 15 d with individual housing in cages with running wheels. During the last 5 d of running, the eight animals on escitalopram ran significantly less than the corresponding eight animals on vehicle (4.2 ± 1.0 km vs. 10.2 ± 2.4 km, respectively, Student's t test $p=0.04$), thus confirming the original finding that escitalopram decreases running in FSL rats.

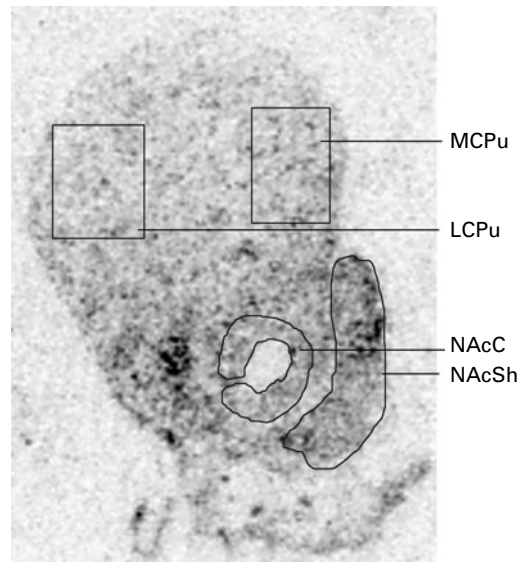


Figure 4. Quantitative computerized image analysis was performed over the indicated areas. Analysis was performed at the level of Bregma 1.30–1.60 mm. LCPu, lateral caudate putamen; MCPu, medial caudate putamen; NAcSh, nucleus accumbens shell; NAcC, nucleus accumbens core.

Behaviour in the PST

On experimental day 42 all FSL rats were subjected to a modified version of the PST to investigate the effect of escitalopram (Esc + nRW), access to running wheels (Veh + RW), and a combination of these two conditions (Esc + RW) on swimming and climbing behaviour. The Veh + RW group had increased climbing but no effect on swimming, whereas the Esc + RW group had increased swimming but no change in climbing compared to the Veh + nRW control group ($p < 0.01$) (Figure 3).

Effects of escitalopram diet, running-wheel access, and their combined effect on mRNAs encoding DA D_1 and D_2 receptors, neuropeptide Y, proenkephalin, prodynorphin, substance P and neurokinin A in FSL rats

Analysis of mRNAs encoding DA D_1 and D_2 and neuropeptide Y, proenkephalin, prodynorphin, substance P and neurokinin A was performed in the medial and lateral caudate putamen as well as in the NAc core and shell (Figure 4). The combined treatment (Esc + RW) significantly decreased D_1 receptor mRNA levels in the NAc shell ($p < 0.01$) and had a strong trend to a decrease in core and lateral caudate putamen ($p=0.06$) (Figure 5 and Table 2) compared to control animals (Veh + nRW). Interestingly, escitalopram treatment without running-wheel access did not affect DA D_1 receptor mRNA levels compared to

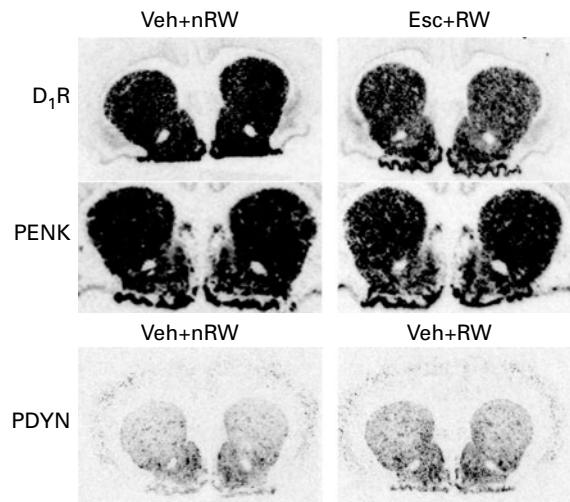


Figure 5. Dopamine D₁ receptor (D₁R), proenkephalin and prodynorphin mRNA expression. In-situ autoradiogram of DA D₁ receptor, proenkephalin (PENK) and prodynorphin (PDYN) mRNA expression at the level of Bregma 1.60 mm in Flinders Sensitive Line (FSL) rats on vehicle diet and no running wheel (Veh + nRW) and FSL rats on escitalopram diet with running-wheel access (Esc + RW). Note the decrease in the nucleus accumbens shell (NAcSh) region in DA D₁R with the combined escitalopram and running treatment. PENK mRNA was decreased in the NAcSh after the combined escitalopram and running treatment. Running increased mRNAs encoding PDYN levels in medial caudate putamen. (For more detailed information on mRNA levels, see Table 2.)

vehicle controls. Moreover, comparing the two treatments with escitalopram diet, DA D₁ receptor mRNA levels were decreased in the combined Esc + RW group compared to the Esc + nRW groups in lateral caudate putamen ($p < 0.05$).

Prodynorphin mRNA was elevated by running (Veh + RW) in the medial caudate putamen ($p < 0.05$) and there was a trend towards elevated levels in lateral caudate putamen and NAc shell compared to controls (Veh + nRW) ($p = 0.06$) (Figure 5 and Table 2).

Proenkephalin mRNA levels were lower in the combined treatment group (Esc + RW) compared to controls (Veh + nRW) in the NAc shell ($p < 0.05$), with a trend to a decrease in the core (Figure 5 and Table 2). The mRNA levels of the DA D₂ receptor, substance P and neurokinin A and neuropeptide Y were unaffected by all treatment conditions (data not shown).

The effect of treatments on BDNF mRNA in the hippocampus

The neurotrophic factor, BDNF, is involved in neuronal plasticity and survival, and suggested to be

important in antidepressant treatments (Altar, 1999; Dias et al., 2003; Nibuya et al., 1995). BDNF mRNA was analysed in the pyramidal cells of CA1, CA3 and CA4/hilus as well as in dentate gyrus (Figure 6). Chronic treatment with escitalopram did not affect BDNF mRNA expression compared to control animals on vehicle diet. Running elevated BDNF mRNA and in CA1, CA3 and CA4 ($p < 0.05$ – 0.01). No effect of the combined treatment was found (Figure 7, Table 2).

To investigate possible connections between different swim test parameters (swimming and climbing), and regional mRNA expression of BDNF, DA D₁ receptor, enkephalin and dynorphin mRNAs Pearson's product moment correlations were performed. Climbing behaviour was positively correlated to BDNF in the CA3 region ($r = 0.44$, $p < 0.05$, $n = 32$) (Figure 7).

Discussion

Exercise has an antidepressive effect in humans (Babyak et al., 2000; Martinsen et al., 1985) and recently we showed that running also has an antidepressive-like effect in 'depressed' FSL rats (Bjørnebekk et al., 2005; Brené et al., 2007). In this study, we compared the antidepressant effects of exercise to that of escitalopram and analysed whether the two treatments have an additive effect in an animal model of depression.

Escitalopram affects running behaviour

There is evidence that different antidepressant compounds can exert a specific effect on running behaviour; for instance, fluoxetine but not imipramine decreases running in an animal model of anorexia and obsessive-compulsive disorder (Altemus et al., 1996). In the present study we demonstrated that chronic escitalopram treatment inhibited voluntary running in female FSL but not SD rats at a calculated average daily dose of 27.5 mg/kg. One possibility is that escitalopram decreased overall motor activity in the FSL strain. However, in the PST the FSL rats on the escitalopram diet with running-wheel access had increased swimming behaviour, which would suggest that escitalopram at our given dose does not inhibit general motor activity. In female mice a decrease in running activity by another SSRI-type drug fluoxetine is dose-dependent (Engesser-Cesar et al., 2007). Thus, escitalopram appears to be able to block one motor behaviour, wheel running, but not another, swimming, in female FSL rats. We cannot rule out that the attenuation of running by escitalopram at a given dose can be a strain-specific trait and that a higher dose of

Table 2. Dopamine D₁ receptor (D₁R), prodynorphin, proenkephalin and BDNF mRNA levels in FSL rats after different antidepressant treatments

	Veh + nRW	Esc + nRW	Veh + RW	Esc + RW
Dopamine D₁R				
LCPu	96 ± 5	101 ± 4.5	100 ± 4.8	80 ± 6.4***
MCPu	87 ± 4.7	94 ± 3.3	94 ± 4.7	78 ± 6.2
NAc shell	103 ± 3.5	94 ± 7.4	106 ± 4.6	73 ± 5.3**
NAc core	73 ± 4.5	71 ± 3.4	77 ± 3.3	62 ± 3
Prodynorphin				
LCPu	21 ± 0.8	20 ± 0.5	25 ± 2.1	18 ± 1.3
MCPu	20 ± 0.4	20 ± 0.7	25 ± 2*	19 ± 1*
NAc shell	54 ± 1.6	57 ± 2	60 ± 2.9	52 ± 2.8
NAc core	39 ± 3.5	35 ± 2.3	47 ± 5	34 ± 3.3
Proenkephalin				
LCPu	131 ± 2.7	135 ± 5.5	124 ± 6	124 ± 6.9
MCPu	133 ± 3	133 ± 6	123 ± 4	123 ± 3
NAc shell	96 ± 4.8	88 ± 5.6	90 ± 3.6	76 ± 3**
NAc core	114 ± 4.6	107 ± 5.9	108 ± 5.9	99 ± 2.3
BDNF				
CA1	7 ± 1.6	9 ± 2.0	13 ± 1.4*	10 ± 1.0
CA3	32 ± 2.8	32 ± 3.4	41 ± 2.8*	32 ± 1.4
CA4	22 ± 2.0	23 ± 3.2	33 ± 2.7**	22 ± 0.8
DG	39 ± 4.2	43 ± 4.5	46 ± 4.3	46 ± 4.0

Veh + nRW, Vehicle + no running wheel; Esc + nRW, escitalopram + no running wheel; Veh + RW, vehicle + running wheel; Esc + RW, escitalopram + running wheel. LCPu, Lateral caudate putamen; MCPu, medial caudate putamen; NAc shell, nucleus accumbens shell; NAc core, nucleus accumbens core; CA1–4, fields of Ammon's horn; DG, dentate gyrus.

Levels of dopamine D₁ receptor, prodynorphin, and proenkephalin mRNA in ventral and dorsal striatum and BDNF mRNA in hippocampus (represented as optical densities) were quantified and are shown as mean nCi/g ± s.e.m. FSL rats were individually housed and divided into four subgroups receiving different treatments with eight animals per group.

*, ** Indicates mRNA differences between the Veh + nRW group and a treatment group (Esc + nRW, Veh + RW or Esc + RW).

*** Indicates differences between Esc + nRW vs. Esc + RW.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.05$. Bold values highlight statistically significant results.

escitalopram could attenuate wheel running in a similar way in the SD strain as in the FSL rats. The striatum receives efferents from cerebral cortex, and makes a loop via basal ganglia output nuclei and thalamus, back to the cortex (Gerfen, 1992; Gerfen et al., 1982). The 'direct' striatonigral pathway conveys the output of striatum, which sends axons directly to substantia nigra, and the 'indirect' pathway, which via the globus pallidus and subthalamic nuclei projects to the substantia nigra and internal pallidum. Stimulation of D₁ receptors located on GABAergic medium spiny neurons facilitates activity in the striatonigral pathway, which generates release of the inhibitory neurotransmitter GABA in target nuclei (You et al., 1994) and thus disinhibits thalamocortical activation (Albin et al., 1989). Interestingly, DA D₁ receptor mRNA was decreased in NAc shell and showed a trend to decrease in core and lateral caudate putamen in animals administered escitalopram with

running-wheel access. Conceivably, a decrease in D₁ receptor levels would lead to less activation in the direct pathway. Since the direct pathway is important for controlling locomotion it is possible that decreased activity in this pathway could explain the decreased running in FSL rats after escitalopram.

Differential responses in the PST by running and escitalopram

Studies in human subjects have shown that physical exercise can attenuate depressive symptoms (Blumenthal et al., 1999), and in some cases is even more efficient in preventing depressive relapses than antidepressive medication (Babyak et al., 2000). We recently demonstrated that running decreases immobility and thus has an antidepressant-like effect in male 'depressed' FSL rats (Bjørnebekk et al., 2005). To further assess the antidepressive potency

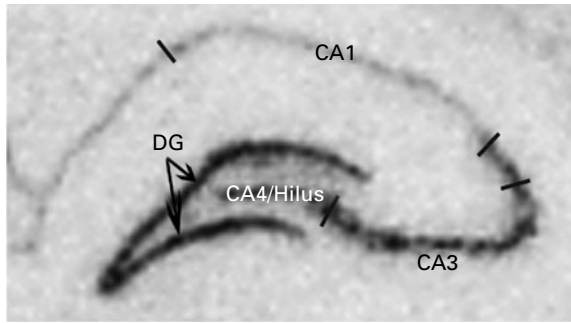


Figure 6. Quantitative computerized image analysis was performed over the indicated areas. Analysis was performed approximately at the level of Bregma -3.30 mm. CA1-4, Fields of Ammon's horn; DG, dentate gyrus.

of escitalopram, running, and the combination of the two, we analysed swimming and climbing behaviour in a modified version of the PST. Typically, SSRI-type drugs enhance swimming (Caberlotto et al., 1999; El Khoury et al., 2006; Jimenez-Vasquez et al., 2007), whereas drugs that act upon norepinephrine transmission enhance climbing (Detke et al., 1995). Running-wheel access in rats on vehicle diet increased climbing, whereas animals on escitalopram diet had increased swimming but no change in climbing behaviour. Thus, running appears to have a norepinephrine antidepressant-like response whereas escitalopram with running-wheel access had a serotonergic antidepressant-like response.

Escitalopram treatment alone did not significantly increase swimming, which is at variance with earlier reports on the effect of citalopram and other SSRI compounds (El Khoury et al., 2006; Hemby et al., 1997; Overstreet et al., 2004; Reneric and Lucki, 1998; Sanchez et al., 2003). In fact, group-housed FSL individuals from the same colony with a similar dose of escitalopram indeed increased swimming in the PST (El Khoury et al., 2006). Housing conditions (Åberg et al., 2005; D'Arbe et al., 2002; Plaznik et al., 1993) and gender (Bjørnebekk et al., 2007a; Westebroek et al., 2004) are important factors that affect the response in different experimental models. In the present study, individually housed female rats on escitalopram diet did not significantly increase the swimming time in the PST. However, the addition of a running wheel to the cage facilitated swimming behaviour. FSL rats with escitalopram both from the groups with and without running wheels increased in body weight whereas the FSL rats on vehicle diet did not. It is conceivable, although not very likely, that weight alterations caused by the escitalopram diet is a factor that contributes to the behaviour in the PST. One reasonable interpretation of the data is that

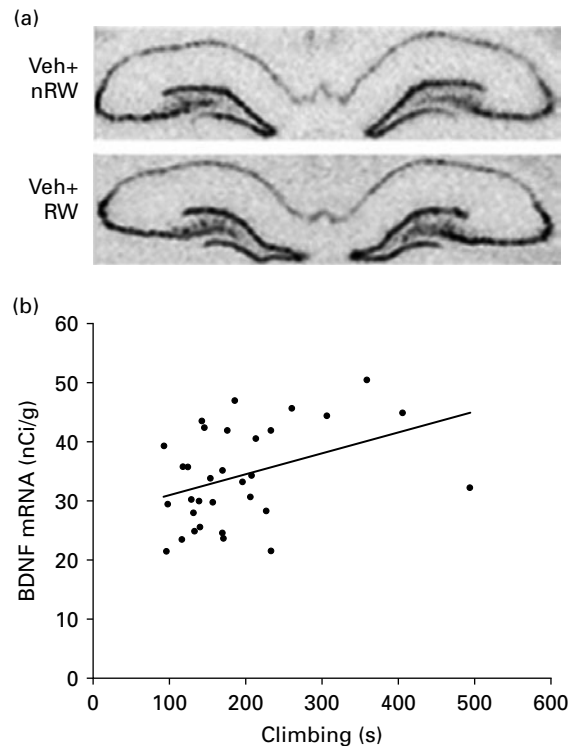


Figure 7. Brain-derived neurotrophic factor (BDNF) mRNA expression in hippocampus in Flinders Sensitive Line (FSL) rats with running-wheel access. (a) In-situ autoradiogram of BDNF mRNA in hippocampus in FSL rats on vehicle diet with or without running-wheel access (Veh + NrW; Veh + RW). Note the increased BDNF mRNA expression in CA1, CA3 and CA4 in the group of runners. (b) Illustration of correlations of BDNF mRNA levels in the CA3 region of hippocampus to climbing behaviour in the Porsolt swim test ($r=0.40$, $p<0.05$, $n=32$).

escitalopram treatment alone, at least at the dosage given, was not sufficient to counteract both the genetic loading and the environmental conditions that may reinforce the depressed FSL phenotype. However, effects of escitalopram on swimming behaviour in individually housed FSL rats were manifested in rats that had a running wheel in their cages. Since FSL rats on escitalopram displayed only a minimal running activity, it seems plausible that the effects of escitalopram are not due to the running activity, but rather to the enriched housing situation, which facilitated the escitalopram effects. Thus, both the socially housed female FSL rats (El Khoury et al., 2006), and the individually housed FSL rats with enriched housing and running wheel, respond to the same dose of escitalopram with increased swimming in the PST whereas socially deprived non-enriched animals do not. This is consistent with findings that the long-term outcome for depressed patients is better for those treated with a

combination of antidepressant drugs and psychotherapy compared to drug alone (Frank et al., 2005; Kupfer and Frank, 2001; Middleton et al., 2005).

The combined escitalopram running-wheel treatment regulates DA D₁ receptor and proenkephalin mRNAs

In the present study, only the combination of escitalopram and running-wheel access significantly increased swimming behaviour in the PST. Likewise, the combination of running-wheel and escitalopram treatment decreased DA D₁ and proenkephalin mRNA in NAc shell. The findings of decrease in proenkephalin in NAc after escitalopram confirm previous investigations demonstrating that chronic diet with different classes of antidepressants decreases met-enkephalin immunoreactivity in the NAc in rats (Kurumaji et al., 1988), suggesting a possible contribution of enkephalin decrease in NAc to the mechanism of antidepressant action. However, others have shown antidepressant-like effects of delta-opioid receptor agonists (Broom et al., 2002) and enkephalinase inhibitors (Tejedor-Real et al., 1998). Interestingly, animals lacking enkephalin do not show a depressive-like phenotype in the PST or the tail suspension test. Moreover, there is no difference in the efficacy of antidepressant compounds in wild-type and proenkephalin knockout mice, suggesting that enkephalin is not necessary to achieve an antidepressant-like effect (Bilkei-Gorzo et al., 2007). Consequently, in spite of some conflicting results, the present findings of down-regulation of proenkephalin and the down-regulation of met-enkephalin immunoreactivity in the NAc after different antidepressant compounds suggest possible involvement of enkephalin in the antidepressant-like effect of escitalopram.

As previously mentioned the combined treatment was also associated with a decrease in DA D₁ receptor mRNA levels in NAc shell. Previous investigations have shown that different antidepressant compounds differentially regulate mRNA levels of the D₁ receptor (Dziedzicka-Wasylewska et al., 1997). The DA D₁ receptor in striatum is essential for voluntary motor activity and mediation of reward. Thus, the decrease in D₁ receptor mRNA found in animals after escitalopram diet with running-wheel access is probably more involved in the regulation of motor activity than in the antidepressant-like effect of escitalopram.

Running elevates prodynorphin mRNA levels

Expression of mRNA encoding the striatal neuropeptides proenkephalin, prodynorphin and substance P is partly regulated by DA (Gerfen et al., 1991). In the

present study prodynorphin mRNA was increased in the medial caudate putamen after running. This is in line with previous reports of prodynorphin mRNA increases in the caudate putamen and striatum after running and ingestion of drugs of abuse (Bjørnebekk et al., 2005; Hurd et al., 1992; Hurd and Herkenham, 1992; Werme et al., 2000). Stimulation of dynorphinergic kappa receptors on presynaptic DA terminals in NAc is suggested to decrease DA release and to underlie a dysphoric mood state (Pfeiffer et al., 1986; Pliakas et al., 2001). It is possible that the increased dynorphin levels in the group of runners constitute a mechanism to protect the animals from developing a compulsive running behaviour.

A possible role of hippocampal BDNF in the antidepressant-like effect of running

Trophic factors are suggested to have an important role for adaptive changes and the trophic factor BDNF is important for the development of learning, memory and LTP in hippocampus (Poo, 2001; Taylor et al., 1985). Chronic administration of variant classes of antidepressant drugs, electroconvulsive seizures (ECS) and physical exercise increase the expression of BDNF and its receptor, trkB mRNA, in hippocampal regions (Neeper et al., 1996; Nibuya et al., 1995), and BDNF is implied to have a role in control of plastic changes induced by antidepressant treatments (Duman, 2004). However, the BDNF hypothesis of antidepressant action has been questioned by a number of findings. Chronic fluoxetine treatment increases (Nibuya et al., 1996), decreases (Miro et al., 2002), or has no effect (Conti et al., 2002; Dias et al., 2003) on BDNF mRNA levels in hippocampus. In the present study, BDNF mRNA was elevated in the CA1, CA3 and CA4 region in FSL runners on the vehicle diet but not in the group of animals on the escitalopram diet with a running-wheel that was not used for running. In a previous study we showed that hippocampal BDNF mRNA levels were not increased in FSL rats with running-wheel access (Bjørnebekk et al., 2005). However, since hippocampal BDNF mRNA levels are correlated to running activity (Bjørnebekk et al., 2005; Neeper et al., 1996; Widenfalk et al., 1999) and the runners in the present study had a higher running activity, the discrepancy between the two studies can possibly be explained by the fact that the FSL rats on vehicle in the present study on average ran more. BDNF mRNAs were positively correlated to climbing behaviour and antidepressant drugs targeting norepinephrine transmission increase climbing behaviour in the PST (Detke et al., 1995), suggesting a possible role for BDNF in the

antidepressant action of running via modulation of norepinephrine transmission. Interestingly, norepinephrine is increased in several brain regions after chronic physical activity (Dishman et al., 2000; Dunn et al., 1996). Moreover, a systematic lesion of the norepinephrine system followed by 1 wk of voluntary physical activity eliminates the exercise-induced increase of hippocampal BDNF mRNA. Further, a 5-HT lesion does not alter exercise-induced BDNF increase, which suggests that norepinephrine activation is necessary for the enhancement of hippocampal BDNF following exercise, and possible also for the exercise-induced antidepressant-like climbing response in the PST.

The absence of escitalopram-mediated BDNF mRNA regulation in hippocampus confirms a previous study where escitalopram did not affect BDNF mRNA levels, and decreased BDNF protein in hippocampus (Jacobsen and Mork, 2004). Thus, regulation of BDNF levels does not seem to be necessary for the antidepressant response of escitalopram. The hippocampus is both anatomically (Kelley and Domesick, 1982) and functionally connected to the NAc and brain reward pathways. For instance, DA activity in NAc is suggested to modulate input from prefrontal cortex and hippocampus, which is important for goal-directed and motivated behaviours (Grace et al., 2007). Moreover, in rodents the hippocampus is essential for the development-conditioned place preference to the rewarding effects of cocaine (Meyers et al., 2003). Thus, increased hippocampal levels of BDNF, which is important for plasticity, after wheel running is also likely modulate function of brain reward circuitry and thereby also motivated behaviours.

Escitalopram affects weight

Decreased appetite and reduced body weight are features frequently observed in depressed patients according to DSM-IV criteria (APA, 1994) and in 'depressed' FSL rats (Bjørnebekk et al., 2007b). In the present study, there was a substantial weight difference between age-matched FSL and SD rats, the latter weighing about 30% more. The findings of lower body weight in FSL rats is in accordance with previous reports in this strain (El Khoury et al., 2006; Overstreet, 1993; Overstreet et al., 2005) and with weight reduction seen in the maternal separation model of depression (Husum and Mathé, 2002; Penke et al., 2001). In the present study FSL rats on vehicle diet and no running-wheel access did not gain weight during the course of the experiment. There was a tendency for weight increase in the FSL running group on vehicle

diet, albeit not statistically significant. Animals receiving the escitalopram diet increased body weight independently of the presence of the running wheel. This is in line with the weight increase observed in depressed patients following long-term treatment with some SSRIs and is probably due to amelioration of depressive symptoms. In contrast, in the SD strain the weight gain was found in the group with vehicle diet (Veh + RW).

Conclusion

This study demonstrates a novel behavioural strain-specific effect of escitalopram; a selective attenuation of running possibly mediated via a decrease in DA D₁ receptor mRNA levels and less activation in the 'direct' striatonigral pathway. In addition, effects of escitalopram in the PST were enhanced by the presence of a running wheel in the cage, indicating that environmental enrichment can increase the antidepressive effects of escitalopram. We have shown specific effects of running, escitalopram and their combination on DA, enkephalin and BDNF mRNAs in brain reward pathways and hippocampus.

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Statement of Interest

None.

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