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The Antidepressant Effects of Running and Escitalopram Are Associated With Levels of Hippocampal NPY and Y1 Receptor But Not Cell Proliferation in a Rat Model of Depression

Astrid Bjørnebekk,[1] Aleksander A. Mathé,[2] and Stefan Brené[3]*

ABSTRACT: One hypothesis of depression is that it is caused by reduced neuronal plasticity including hippocampal neurogenesis. In this study, we compared the effects of three long-term antidepressant treatments: escitalopram, voluntary running, and their combination on hippocampal cell proliferation, NPY and the NPY-Y1 receptor mRNAs, targets assumed to be important for hippocampal plasticity and mood disorders. An animal model of depression, the Flinders Sensitive Line (FSL) rat, was used and female rats were chosen because the majority of the depressed population is females. We investigated if these treatments were correlated to immobility, swimming, and climbing behaviors, which are associated with an overall, serotonergic-like and noradrenergic-like antidepressant response, in the Porsolt swim test (PST). Interestingly, while escitalopram, running and their combination increased the number of hippocampal BrdU immunoreactive cells, the antidepressant-like effect was only detected in the running group and the group with access both to running wheel and escitalopram. Hippocampal NPY mRNA and the NPY-Y1 receptor mRNA were elevated by running and the combined treatment. Moreover, correlations were detected between NPY mRNA levels and climbing and cell proliferation and NPY-Y1 receptor mRNA levels and swimming. Our results suggest that increased cell proliferation is not necessarily associated with an antidepressant effect. However, treatments that were associated with an antidepressant-like effect did regulate hippocampal levels of mRNAs encoding NPY and/or the NPY-Y1 receptor and support the notion that NPY can stimulate cell proliferation and induce an antidepressant-like response. © 2009 Wiley-Liss, Inc.

KEY WORDS: depression; SSRI; neurogenesis; exercise; neuropeptide Y

INTRODUCTION

Reduced hippocampal volume has been reported in patients diagnosed with major depression (Sheline et al., 1996; Bremner et al., 2000; Campbell et al., 2004). The hippocampal formation is also one of the two major regions in the adult brain where new neurons are formed (Altman, 1962). Decreased neurogenesis has so far not been demonstrated in depressed patients and the hypothesis that reduced neuronal plasticity and disturbed hippocampal neurogenesis (Duman et al., 1999) can account for depression pathophysiology (Duman et al., 1999) is mostly based on findings from healthy rodents exposed to stress. We have previously found adult cell proliferation to be lower in single housed Flinders Sensitive Line (FSL) rats, an animal model of depression, compared to single housed Flinders Resistant Line (FRL) control strain (Bjørnebekk et al., 2005). Other studies using a BrdU-administration paradigm to study survival of proliferated cells in socially housed and younger FSL animals (Husum et al., 2006; Petersén et al., 2009) have shown a higher number of BrdU positive cells in the dentate gyrus (DG). Findings that neurogenesis is increased in rodents by virtually all treatments that have an antidepressant effect in humans, such as electroconvulsive stimuli (ECS) and pharmacological treatments with selective serotonin reuptake inhibitors (SSRIs), tricyclics, and lithium (Chen et al., 2000; Madsen et al., 2000; Malberg et al., 2000; Hellsten et al., 2002; Santarelli et al., 2003) have been taken as further evidence for the decreased neurogenesis hypothesis of depression. Recently, we reported that exercise, which has an antidepressant effect in patients (Blumenthal et al., 1999; Babyak et al., 2000; Wong and Licinio, 2001), decreases immobility in the Porsolt Swim Test (PST) (Bjørnebekk et al., 2005; Bjørnebekk et al., 2008) and is associated with increased cell proliferation in the dentate gyrus as well as increased NPY mRNA in FSL rats (Bjørnebekk et al., 2005, 2006). Contradictory results where depressive-like behavior occurs without impaired neurogenesis (Vollmayr et al., 2003; Li et al., 2008; Petersén et al., 2009), and where SSRI, tricyclics, and melanin-concentrating hormone receptor antagonist produce antidepressant-like effects without increasing the rate of neurogenesis (Vollmayr et al., 2003; Howell et al., 2006; Meshi et al., 2006; David et al., 2007; Holick et al., 2008; Huang et al., 2008; Petersén et al., 2009) argue against that levels and regulation of hippocampal neurogenesis is directly linked to mood and required for antidepressant treatments.

[1] **Department of Neuroscience, Karolinska Institute, Stockholm, Sweden;**
[2] **Karolinska Institute, Department of Clinical Neuroscience, Psychiatry M56, Karolinska University Hospital Huddinge, Stockholm, Sweden;**
[3] **Department of Neurobiology, Care Sciences and Society, Karolinska University Hospital Huddinge, Stockholm, Sweden**
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*Correspondence to: Stefan Brené, Karolinska Institute, Department of Neurobiology, Care Sciences and Society, Karolinska University Hospital Huddinge, Stockholm S-141 86, Sweden. E-mail: Stefan.Brene@ki.se
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Neuropeptide Y (NPY) is widely distributed within the nervous system, with high concentration in several limbic and cortical regions. Clinical and experimental data suggest a role for NPY in the pathophysiology of depression (Mathé, 1999; Stogner and Holmes, 2000; Heilig et al., 2004; Mathé and Gruber, 2004). NPY levels are decreased in cerebrospinal fluid (Heilig et al., 2004) and plasma (Nilsson et al., 1996) of depressed patients, and in postmortem brain tissue from suicide victims (Widdowson et al., 1992). In genetic and environmental rat models of depression, NPY is decreased in hippocampus (Mathé et al., 1998; Bjørnebekk et al., 2006; Jimenez-Vasquez et al., 2000a,b, 2001, 2007) and, conversely, enhancement of the NPYergic transmission is a mechanism that is shared by antidepressants and electroconvulsive therapy (Mathé et al., 1996; Mathé, 1999; Husum et al., 2002; Nikisch et al., 2005; Nikisch and Mathé, 2008). Infusions of NPY or an NPY-Y1 agonist into the cerebral ventricle or the CA3 region induces antidepressant-like effect in the PST, which is blocked by coadministration of a NPY-Y1 antagonist (Stogner and Holmes, 2000; Redrobe et al., 2002; Mathé and Gruber, 2004; Goyal et al., 2006; Ishida et al., 2007). Moreover, a role for NPY in promoting proliferation of neuronal precursor cells, possibly mediated by the Y1 receptor has been suggested (Hansel et al., 2001; Howell et al., 2005; Bjørnebekk et al., 2006).

In this study, we compared the effects of escitalopram, wheel-running and a combination of these treatments on mRNA regulation of NPY and the NPY-Y1 receptor. We also analyzed how the different treatments affect the number of proliferated cells that were labeled 14–24 days prior to the end of the experiment and survived until its end. Moreover, we correlated effects of treatments to behavior in the PST to identify possible mechanisms that contribute to the understanding of how different treatments might relieve depressive symptoms presumably related to hippocampal abnormalities in major depression.

MATERIALS AND METHODS

Animals

Female FSL rats ($n = 32$) bred at the Karolinska Institute were used. The rats were 22-weeks old at the beginning of the experiment. They were individually housed throughout the experiment in cages (43 cm × 22 cm × 20 cm) with ($n = 16$) or without ($n = 16$) access to running-wheels. During the first 14 days of the experiment all rats had identical cages. On day 14, running wheels were placed in half of the cages (Fig. 1). The animals were divided into four groups of eight rats each: (1) vehicle diet (Veh), (2) escitalopram diet (Esc), (3) vehicle diet and running wheel in the cage (Veh + Run), (4) escitalopram diet and running wheel in the cage (Esc + Run). Rats had access to food and water ad libitum and were subjected to a controlled 12 h light:12 h dark-schedule (lights on at 07.00 h). The Ethical Committee for Animal Research in Stockholm approved the experiments.

Administration of Escitalopram

Administration of escitalopram or vehicle in pellets started 14 days prior to access to the running wheels and continued throughout the whole experiment (Fig. 1) according to a method developed by H Lundbeck A/S (Copenhagen, Denmark) and tested in collaboration with A. Mørk (El Khoury et al., 2006). The experiment was run in two consecutive steps, each with 16 rats, which includes the two groups on escitalopram (Esc, Esc + Run). The escitalopram doses were 0.35 g 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/day was calculated. After decapitation, trunk-blood samples were collected and serum levels of escitalopram were measured.

Wheel Running

Half of the rats had access to running wheels during 31 days (diameter 34 cm; one revolution corresponding to 1.07 m) (Werme et al., 1999, 2000) (Fig. 1). Running data were sampled 48 times/day using a computer-based data system with customized software.

Porsolt Swim Test

A modified swim test was performed (Bjørnebekk et al., 2005; Cryan et al., 2005a,b) on day 42 (Fig. 1). Transparent cylinders (24 cm diameter) filled with water at 25°C to the level that prevents the animals from reaching the bottom of the cylinder 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 analyzed by an observer blind to the treatment condition. Immobility was defined as a stationary posture when the only movements of the rat are those necessary to keep the head above the water. At least three of the rat's paws had to be immobile. 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 killed on day 45, 3 days after the swim test (Fig. 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.

BrdU Administration

To evaluate cell survival, 5-bromo-2deoxyuridine (BrdU) was administered in the drinking water (1 mg BrdU/ml water, Sigma) during 10 days starting on day 21 of the experiment (Bjørnebekk et al., 2007). With this administration schedule, new hippocampal cells are labeled during all the different stages of the estrous cycle. Because the estrous cycle of rats lasts for

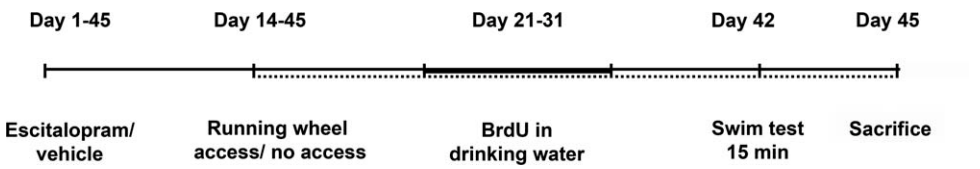

FIGURE 1. A schematic illustration of the experimental design. On day 1, the animals were started on either escitalopram ($n = 16$) or vehicle diet ($n = 16$). From day 14 to the end of the experiment (day 45), half of the rats were single housed in cages containing a running wheel ($n = 16$). Bromodeoxyuridine (BrdU) was administered in the drinking water (1 mg BrdU/ml water) from day 21 to 31. On day 42, a modified Porsolt Swim Test was performed ($n = 32$). Animals were killed on day 45.

4–5 days by administering BrdU for 10 days most of the potential variability caused by the estrous phase is eliminated. The intake of BrdU containing water was measured three times during the 10-day period and mg/BrdU/kg body wt/day was calculated. After 5 days, the animals received fresh BrdU containing water with equal concentration.

BrdU Immunohistochemistry

For the immunohistochemistry, coronal 30 μm sections were collected with a cryostat throughout the hippocampal formation. Antibodies and dilutions used were as follows: mouse α-BrdU (1:100 DAKO A/S, Denmark), horse α-mouse-biotin (1:200 Vector, Burlingame, CA, USA). Immunohistochemistry for BrdU was performed as follows: sections were taken out of freezer (−20°C) and post-fixed for 10 min in 4% formaldehyde, rinsed in phosphate buffered saline (PBS) 4 × 5 min, incubated 30 min in 2 M HCl at 37°C, rinsed 3 × 5 min in PBS, and incubated for 1 h in blocking solution (horse serum 10%, 0.1% tween in PBS) at room temperature. This 1 h incubation was followed by overnight incubation with mouse α-BrdU at 4°C. On day 2, the samples were rinsed 3 × 30 min in 0.1% tween PBS, incubated with horse α-mouse-biotin for 60 min at room temperature, rinsed again for 90 min in PBS 0.1% tween followed by 30 min in PBS only. The sections were then incubated for 40 min at room temperature with avidin-biotin-peroxidase complex (1:100 in PBS, Vectastain Elite, Vector, Burlingame, CA), then rinsed in PBS for 1 h, followed by peroxidase detection (0.7 mg/ml, DAB dissolved in H_2O) (DAB Peroxidase Substrate, Sigma) for about 25 s per section. The sections were rinsed in PBS and stained with a hematoxylin solution (Vector).

Stereology of BrdU Positive Cells

For quantification of BrdU positive cells in the dentate gyrus the unbiased optical fractionater counting procedure was performed (West et al., 1991). Coronal sections of 30-μm were taken throughout the hippocampus and every fifteenth section (450-μm apart) was selected for analysis of the right dentate gyrus. An unbiased counting frame with known area was superimposed on the field of view by appropriate software (Stereologer™, SPA). The counting frames were systematically distributed with known x and y steps throughout the marked region from a random starting point. The area of the counting frame relative to the area associated with the x and y step gives the second fraction (area sampling fraction [asf]). The height of the optical dissector relative to the thickness of the section results in the third fraction (height [h]/thickness [h]). The total number of neurons is given by $N\text{total} = \sum Q - \cdot \frac{1}{\text{ssf}} \cdot \frac{1}{\text{asf}} \cdot \frac{t}{h}$ where $\sum Q-$ is the number of neurons counted in the dissectors. The dentate gyrus was manually outlined using a 10× lens. Cell counts were performed with a 60× lens (numerical aperture = 1.4). Positive cells were counted if they where within the dissectors. Cells situated further than two cell body widths away from the base of the granular cell layer were defined as belonging to hilus, and thus not counted. Also, cells were excluded if they were situated in the lowermost focal plane. To estimate total number of BrdU cells per individual, a representative material of BrdU immunoreactive cells in the dentate gyrus of the left hemispheres was compared to that of the right hemispheres. T-tests showed that there were no differences in number of BrdU immunoreactive cells between the two hemispheres, and the total number of cells per individual was calculated.

In Situ Hybridization

Coronal brain sections (30 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 Na_3PO_4, 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 NPY (nt 1671–1714) (Larhammar et al., 1987) and NPY-Y1 receptor (nt 141–188, in rat NPY R sequence in gene bank under accession no NM001013032.1) were used. The probes were 3′-end labeled with α-^{33}P-dATP (Perkin-Elmer, Boston, MA) to a specific activity of approximately 1×10^9 cpm/mg using terminal deoxynucleotidyl transferase (Gibco). 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) for 5–29 days before being developed. Films were scanned and optical density values quantified using appropriate software (NIH image analysis program, version 1.62). A ^{14}C step standard (Amersham, Buckinghamshire, UK) was

included to calibrate optical density readings and convert measured values into nCi/g.

Statistical Analyses

Two-way ANOVAS/MANOVAS with planned comparison post-hoc tests were performed to analyze effects of treatments on immobility, cell survival, and mRNA expression of NPY and the NPY-Y1 receptors in regions of interest. To compute correlations between variables, Pearson Product-Moment Correlation was performed (Statistica; v.99, StatSoft, Tulsa, USA).

RESULTS

Immobility time in the PST and neurochemical adaptive responses to different antidepressant treatments were analyzed at baseline (Veh) and after chronic administration of escitalopram (Esc), 1 month access to running-wheels (Veh + Run), and a combination of escitalopram administration and running-wheel access (Esc + Run). BrdU immunoreactive cells and mRNAs encoding NPY and the NPY-Y1 receptor were analyzed in hippocampal subregions (Fig. 2). Data on animal weight, escitalopram intake, and serum levels have previously been reported (Bjørnebekk et al., 2008). Briefly, all treatment groups gained weight during the course of the experiment, whereas the vehicle control group did not. Mean escitalopram intake was calculated to 27.5 ± 2.5 mg Esc/kg body weight, and mean escitalopram serum level was 26.7 ± 6.7 ng/ml, and did not differ between the two groups receiving escitalopram pellets.

The Effect of Treatments on Immobility in the Porsolt Swim Test

The effects of the different chronic treatments on immobility behavior in the PST were examined. Immobility time was reduced by four weeks of voluntary running (Veh+Run) ($P < 0.05$), and by the combined treatment (Veh + Esc) ($P < 0.001$) (Table 1). It is noteworthy that running behavior was minimal in the Veh + Esc group (Bjørnebekk et al., 2008). For data on swimming and climbing behavior see Table 1, reviewing PST data from Bjørnebekk et al. (2008).

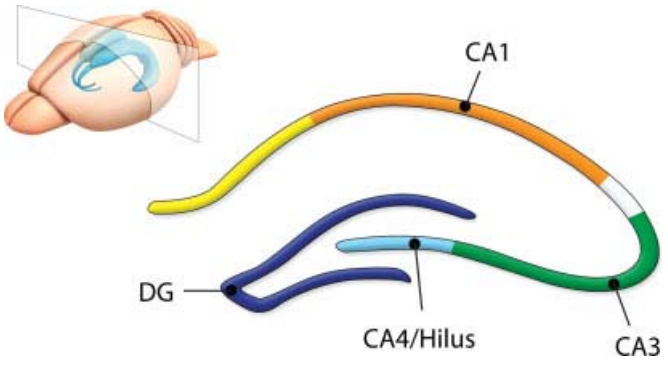

FIGURE 2. Quantitative computerized image analysis was performed over the indicated areas. Analyses were performed approximately at the level of Bregma −3.30 mm. CA1–4, fields of Ammon's horn; DG, dentate gyrus. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 1.

Effect of Escitalopram, Voluntary Wheel Running, and a Combination of Escitalopram and Running on Behavior in the Porsolt Swim Test

	Veh	Esc	Veh + Run	Esc + Run
Swimming	172 ± 28	254 ± 49	236 ± 56	400 ± 40**
Climbing	139 ± 11	150 ± 15	269 ± 30**	217 ± 42
Immobility	437 ± 30	388 ± 65	279 ± 52*	183 ± 39***

The table illustrates the time (in s) FSL rats engaged in swimming, climbing and immobility behavior after different antidepressant treatments. Values are presented as mean ± SEM. The treatments conditions were as follows: vehicle diet without access to running wheel (Veh), escitalopram diet without access to running wheel (Esc), vehicle diet with access to running wheel (Veh + Run), and escitalopram diet with access to running wheel (Esc + Run) ($n = 8$ per group). The rats were placed in cylinders filled with water (25°C) for 15 min. The test was video recorded and analyzed by an observer blind to the experimental conditions. The three treatments had different effect on behavior in the swim test; immobility time was reduced by running (Veh + Run) and the combinded treatment (Esc + Run), time enganged in climbing was increased in the Veh + Run group, and swimming was increased in the Esc+Run group. For more details regarding Porsolt Swim Test data see Bjørnebekk *et al* (2008). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ indicates a significant difference between treatment and the vehicle group.

The Effect of Treatments on Number of BrdU Immunoreactive Cells Labeled 14–24 Days Prior to Sacrifice in the Dentate Gyrus

The number of BrdU labeled cells was higher in the group receiving escitalopram ($P < 0.01$), the running group ($P < 0.001$), and the combined treatment group (Esc+Run) ($P < 0.05$) compared to the group receiving vehicle diet (Fig. 3).

The Effect of Treatments on NPY mRNA in Hippocampus

Administration of escitalopram had no effect on NPY mRNA levels. In contrast, NPY mRNA was elevated after running in all subregions analyzed compared to the vehicle group ($P < 0.001$). In the group receiving the combined treatment, NPY mRNA was also higher than in controls in CA4 and dentate gyrus ($P < 0.01$) (Fig. 4 and Table 2).

Effects of Treatment on Y1 Receptor mRNA in Hippocampus

Running elevated the levels of the Y1 receptor mRNA in the dentate gyrus ($P < 0.01$). In the combined treatment group, the Y1 receptor mRNA was elevated in CA1 and dentate gyrus ($P < 0.05$, 0.01), and there was a trend to elevated levels in

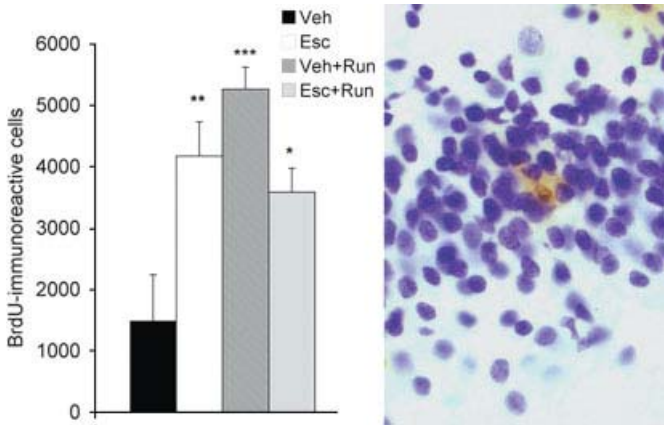

FIGURE 3. Newly proliferated cells in the dentate gyrus in response to different antidepressant treatments. Left panel: Histogram illustrates number of BrdU-immunoreactive cells in the dentate gyrus in female FSL rats on vehicle diet and no access to running wheels (RW), escitalopram diet and no access to RW, vehicle diet and RW access, and escitalopram diet and RW access. All treatment groups increase the number of newly proliferated cells (14- to 24-days old) in the dentate gyrus. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. BrdU, 5-Bromo-2deoxyuridine; FSL, Flinders Sensitive Line; RW, running wheel. Right panel: illustration of a BrdU immunoreactive cell situated in the middle of the granule cell layer of the dentate gyrus. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the group receiving escitalopram treatment without running-wheel access ($P = 0.09$) (Fig. 5 and Table 2).

Correlations of Hippocampal mRNA Levels, Proliferated Cells, and Data From Porsolt Swim Test

For swim test data, see Table 1 reviewing data from the original study (Bjørnebekk et al., 2008). To investigate the possible connections between different swim test parameters (immobility, swimming and climbing), cytogenesis and hippocampal mRNA expression of NPY and the Y1 receptor, Persons Product Moment correlations were calculated. Immobility was negatively correlated with NPY mRNA levels in CA4 and in the dentate gyrus ($r = -0.46$, $r = -0.39$, $P < 0.05$) and with NPY-Y1 receptor mRNA levels in CA1 and in the dentate gyrus ($r = -0.41$, $r = -0.39$, $P < 0.05$, $N = 30$). Climbing was positively correlated with NPY mRNA in all subregions analyzed: CA1, CA4, and dentate gyrus ($r = 0.48$, $r = 0.68$, $r = 0.69$, $P < 0.01$, $N = 30$) (Fig. 5), while swimming was correlated with NPY-Y1 receptor mRNA in the CA1 and dentate gyrus ($r = 0.42$, $r = 0.38$, $P < 0.05$, $N = 30$) (Fig. 6). Interestingly, the only factor that was correlated with increased number of proliferated cells in this study was NPY mRNA in the CA4 region ($r = 0.36$, $P < 0.05$).

DISCUSSION

In this study, we compared the effect of three antidepressant treatments: escitalopram, voluntary wheel-running, and their combination on mRNAs encoding NPY, the NPY-Y1 receptor and cytogenesis in hippocampus, targets assumed to be important for plasticity and mood disorders.

Hippocampal Cell Proliferation Increased After Escitalopram, Running, and the Combined Treatment

Accumulated evidence shows that antidepressant treatments induce several forms of neuroplasticity in the brain (Maya Vetencourt et al., 2008), in particular in hippocampus (Duman et al., 1999; Castren et al., 2007; Christie et al., 2008). Despite increasing insight that exercise improves synaptic plasticity in hippocampus (van Praag et al., 1999; Farmer et al., 2004; Stranahan et al., 2007) and is an antidepressant (Lawlor and Hopker, 2001; Bjørnebekk et al., 2005; Rethorst et al., 2009), there is sparse knowledge about underlying mechanisms shared by exercise and antidepressants and those that differ between them. It is also uncertain whether combining exercise with antidepressant treatment will potentiate the effects of treatments.

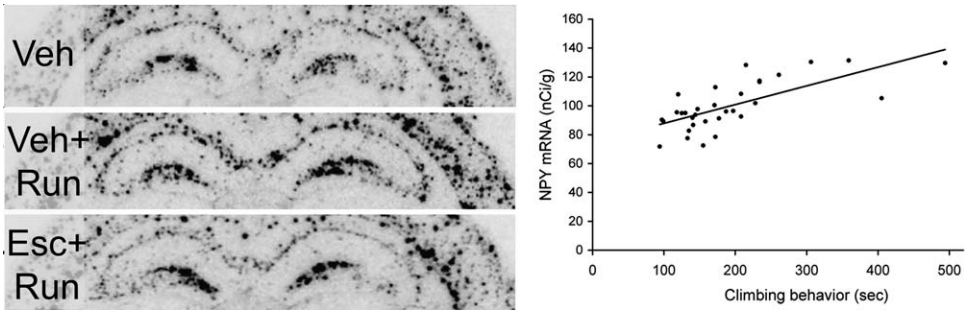

FIGURE 4. NPY mRNA expression in hippocampus after different antidepressant treatments. Left panel: In situ autoradiogram of NPY mRNA in hippocampus in rats on vehicle diet after running (Veh + Run) and combined escitalopram and running treatment (Esc + Run). Running increased NPY mRNA in all areas, and the combined escitalopram and running treatment elevated NPY mRNA in the CA4 and the dentate gyrus. Right panel: NPY mRNA in all regions that were analyzed correlated positively to climbing behavior in the Porsolt Swim Test, here illustrated by a graph showing the correlation between NPY mRNA in the CA4 and time engaged in climbing in a 15 min swim test ($r = 0.68$, $P < 0.001$, $N = 32$).

TABLE 2.

NPY and NPY-Y1 Receptor mRNA Levels After Different Antidepressant Treatments in Single Housed FSL Rats (see Table 1)

	Veh	Esc	Veh + Run	Esc + Run
NPY				
CA1	46.9 ± 0.4	44.5 ± 0.6	60.7 ± 1.0***	48.5 ± 0.8
CA4/hilus	67.9 ± 1.1	74.5 ± 1.4	103.5 ± 1.6***	84.4 ± 2.0**
DG	84.7 ± 1.3	96.3 ± 1.1	115.9 ± 1.8***	102.7 ± 1.9**
Y1rec				
CA1	24.2 ± 2.0	27.3 ± 1.9	27.0 ± 1.5	29.7 ± 1.0*
CA3	15.8 ± 1.4	18.5 ± 1.6	19.6 ± 1.4	21.1 ± 1.3
DG	50.1 ± 3.6	57.8 ± 2.8	63.1 ± 3.1**	63.6 ± 2.2**

Levels of NPY and NPY-Y1 receptor mRNA in hippocampal subregions were quantified and expressed as mean nCi/g ± S.E.M. Analyses were performed approximately at the level of Bregma −3.30. Values are mean ± S.E.M.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ indicate a treatment effect, that is mRNA expression difference between Esc, Veh + Run, or Esc + Run compared to Veh. CA1-4, fields of Ammon's horn; DG, dentate gyrus.

Investigating the behavioral responses by these treatments, we found reduced immobility in the PST after voluntary running and the combined treatment. Recently, we demonstrated that the behavioral antidepressant-like responses to voluntary running and escitalopram in the PST were different; running increased climbing behavior, a response that presumably reflects increased noradrenergic neurotransmission, whereas escitalopram increased amount of swimming (Detke et al., 1995; Cryan et al., 2005a). Interestingly, the antidepressant-like response to escitalopram was only observed in rats housed in a cage that contained a running wheel although it was essentially not used for running (Bjørnebekk et al., 2008).

In this study, all three treatments increased hippocampal cell proliferation supporting the notion that antidepressant treatments enhance plasticity in this region. However, the increase in dentate cytogenesis did not correlate with antidepressant-like behavior in the PST. This is consistent with several recent reports showing a dissociation between neurogenesis and antidepressive effect (Meshi et al., 2006; David et al., 2007; Holick et al., 2008; Huang et al., 2008). The complexity in the regulation of hippocampal neurogenesis is further illustrated by findings that gender, genetic predisposition, and stress response interact and influence cell division. For instance, running induced neurogenesis is dependent on housing condition (single vs. group housed) (Stranahan et al., 2006), strain (Bjørnebekk et al., 2005), and duration of wheel access (Naylor et al., 2005). Moreover, socially isolated female Wistar rats exposed to stress during 3 weeks have increased neurogenesis whereas the same stress paradigm decreased neurogenesis in males (Westenbroek et al., 2004). We recently demonstrated that cell proliferation is increased by social isolation in female FSL rats, whereas the Sprague-Dawley strain is unaffected by housing condition (Bjørnebekk et al., 2007). Our results show that the antidepressive-like effect of escitalopram in the PST was contingent on the housing condition (running wheel in the cage) while, in contrast, neurogenesis was unaffected by that variable. Summarizing, cumulative evidence indicates that antidepressant treatment modalities can affect neurogenesis without modifying behavioral tests assessing antidepressant-like responses and, conversely, can affect antidepressant behavior without changing neurogenesis.

We propose that the increase in neurogenesis observed by the antidepressant treatments is a mechanism that will allow functional modifications of neuronal circuits that are disrupted in mood disorders. However, increased plasticity does not seem to be sufficient to achieve an antidepressant-like response. Optimal functioning of the new neuronal connections is achieved by pruning of synapses where active synapses are stabilized

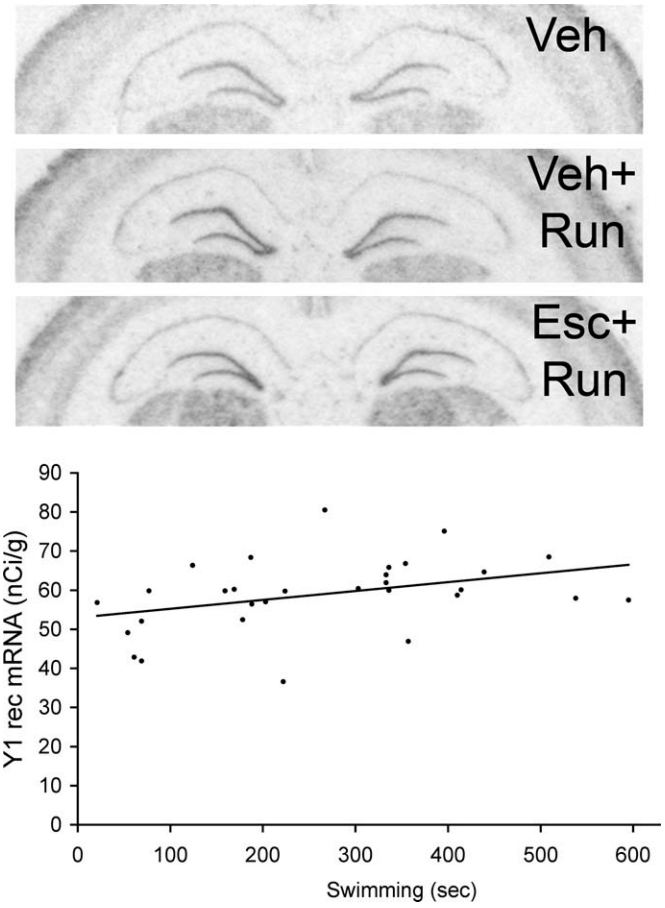

FIGURE 5. Y1 receptor mRNA expression in hippocampus after different antidepressant treatments. Upper panel: In situ autoradiogram of the Y1 receptor mRNA expression in hippocampus in animals on vehicle diet (Veh), on vehicle diet with access to a running wheel (Veh + Run), and on escitalopram diet with access to a running wheel (Esc + Run). Running increased NPY-Y1 receptor mRNA levels in the dentate gyrus, whereas the combined treatment increased NPY-Y1 receptor mRNA levels in the CA1 and in the dentate gyrus. Lower panel: Correlation graph illustrates a positive correlation between NPY-Y1 receptor mRNA levels in the CA1 region and swimming behavior in the Porsolt Swim Test ($r = 0.42$, $P < 0.05$, $N = 30$).

(Castren, 2005). Presumably, in the condition where single housed rats were administered escitalopram, the environment was too impoverished or stressful to utilize the increase in plasticity and improve functioning in pathways important in mood regulations. This could possibly explain why no antidepressant-like response in the PST was found in this group, despite the observed increase in the number of newly proliferated cells.

Recently, we demonstrated that voluntary running has an antidepressant-like effect in FSL rats that is associated with increased NPY and hippocampal cell proliferation (Bjørnebekk et al., 2005, 2006). To elucidate whether the proliferated cells in our running wheel model survive, or whether proliferation is increased but the newly formed cells die shortly after they have been formed, the animals were sacrificed 14 days after the end of a 10-day BrdU treatment. With this paradigm, we observed increased number of BrdU-immunoreactive cells, which suggests that the increase in BrdU immunoreactive cells was due to the fact there is an increased cell proliferation and that the newly proliferated cells also survive for 14–24 days, the most critical period when the majority of the proliferated cells die (Cameron et al., 1993; Gould et al., 1999).

A Possible Role of NPY and the Y1 Receptor in Mediation of Antidepressant-Like Effect

NPY is suggested to mediate an antidepressant effect via activation of NPY Y1 receptor resulting in an increased cell proliferation and neurogenesis in hippocampus. Therefore, we analyzed whether there are any correlations between BrdU labeled cells and the levels of these candidate molecules. The factor that was correlated to BrdU-immunoreactive cell number was NPY mRNA in the hilar region. We have previously demonstrated that level of NPY mRNA in the dentate gyrus and hilus is correlated to the number of BrdU-immunoreactive cells when BrdU is administered 1 day before sacrifice (Bjørnebekk et al., 2006). Data from this study support the notion that NPY may have the potential to stimulate cell proliferation and that the proliferated cells survive for at least 24 days. The neurogenic subgranular zone and the molecular layer of the dentate gyrus are rich in NPY-Y1 receptors (Kopp et al., 2002), and activation of these receptors by NPY released from interneurons is one likely mechanism to activate NPY-Y1 receptors and thereby stimulate neurogenesis (Gruber et al., 1994; Howell et al., 2005). This is consistent with the findings that NPY increases adult neurogenesis in the olfactory bulb (Hansel et al., 2001) and the observed reduction in cell proliferation in the dentate gyrus in knockout NPY (−/−) and NPY-Y1 receptor (−/−) mice (Howell et al., 2005, 2006). Analysis of mRNA levels as in this study only generates an indication of the level of transcription occurring in the cell body and does not provide direct information on when and where the mature peptide is released. In hippocampus NPY peptides that stimulate NPY-Y1 receptors can hypothetically originate from local interneurons. There is also the possibility that the NPY peptide originates from NPY containing noradrenergic terminals in dentate gyrus, with cell bodies in locus coeruleus (Everitt et al., 1984; Köhler et al., 1986)

In this study, NPY mRNA was markedly increased by the treatments that had an antidepressant-like response in the PST, that is running and the combined treatment with escitalopram and running-wheel, barely used for running. The combined treatment increased NPY mRNA expression in the hilus and dentate gyrus, whereas running induced an NPY mRNA increase in all hippocampal regions that were analyzed. This is in line with previous reports suggesting a role for NPY in depression and antidepressant action (Heilig et al., 1988, 1989; Mikkelsen et al., 1994; Mathé et al., 1997; Mathé, 1999; Stogner and Holmes, 2000; Husum et al., 2002; Bjørnebekk et al., 2006; Jimenez-Vasquez et al., 2000a,b, 2007).

CONCLUSION

Our results suggest that both voluntary running and escitalopram treatment induce plasticity in the hippocampus that is related to their antidepressant-like responses in the PST. NPY mRNA levels are significantly increased by antidepressant treatments and correlate to cell proliferation and the climbing responses in the PST confirming and extending the previous results regarding the antidepressant effects of NPY. Moreover, all treatments increased cell proliferation and support the notion that activation of the NPY-Y1 receptor could stimulate cell proliferation and induce an antidepressant-like response.

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