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Running has Differential Effects on NPY, Opiates, and Cell Proliferation in an Animal Model of Depression and Controls

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

[1]Karolinska University Hospital-Huddinge, Division of Psychiatry, Karolinska Institutet, Stockholm, Sweden; [2]Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Physical activity has documented beneficial effect in treatment of depression. Recently, we found an antidepressant-like effect of running in an animal model of depression, the Flinders Sensitive Line (FSL) and demonstrated that it was associated with increased hippocampal cell proliferation. In this study, we analyzed levels of mRNAs encoding the neuropeptide Y (NPY) and the opioid peptides dynorphin and enkephalin in hippocampus and correlated these to cell proliferation in the FSL and in the 'nondepressed' Flinders Resistant Line (FRL) strain, with/without access to running wheels. Running increased NPY mRNA in dentate gyrus and the CA4 region in FSL, but not in FRL rats. NPY mRNA increase was correlated to increased cell proliferation in the subgranular zone of dentate gyrus. Baseline dynorphin and enkephalin mRNA levels in the dentate gyrus were lower in the FSL compared to the FRL strain. Running had no effect on dynorphin and enkephalin mRNAs in the FSL strain but it decreased dynorphin mRNA, and there was a trend to increased enkephalin mRNA in the FRL rats. Thus, it would appear that the CNS effects of running are different in 'depressed' and control animals; modification of NPY, a peptide associated with depression and anxiety, in depressed animals, *vs* effects on opioids, associated with the reward systems, in healthy controls. Our data support the hypothesis that NPY neurotransmission in hippocampus is malfunctioning in depression and that antidepressive treatment, in this case wheel running, will normalize it. In addition, we also show that the increased NPY after running is correlated to increased cell proliferation, which is associated with an antidepressive-like effect.
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INTRODUCTION

Exercise promotes physical health and is also an efficient antidepressant in depressed patients (Blumenthal *et al*, 1999; Martinsen *et al*, 1985; Strawbridge *et al*, 2002) as well as in an animal model of depression (Bjørnebekk *et al*, 2005). In fact, in a recent follow-up study, continued exercise was more efficient in preventing depressive relapses than antidepressant medication (Babyak *et al*, 2000). However, the mechanisms by which exercise produces therapeutic effects are not understood. Running can also be reinforcing. For instance, rodents will lever-press to get access to running wheels and they develop preference for an environment that they associate with the aftereffects of wheel running (Belke, 1997; Iversen, 1993; Lett *et al*, 2001). Interestingly, an opioid receptor antagonist can block this effect, which suggests that endogenous opioid peptides have a role in the reinforcing effects of running in rodents. Moreover, long-term running will cause neuroadaptive changes such as increased ΔFosB and increased dynorphin levels in brain reward pathways that are similar to the adaptations that are seen after chronic administration of addictive drugs (Werme *et al*, 2002, 2000).

Neurogenesis in the adult mammalian brain was described over 40 years ago (Altman, 1962), but remained controversial for a long time (Gross, 2000). The sites of proliferation are well characterized in two areas of the brain, the subventricular zone and in the subgranule zone (SGZ) of the dentate gyrus in hippocampus. Antidepressant treatment modalities such as selective serotonin reuptake inhibitors (SSRI), for example fluoxetine; tricyclics, for example desipramine; and electroconvulsive treatment (ECT), and also running in running wheels, all cause increased cell proliferation and neurogenesis in the SGZ (Fabel *et al*, 2003; Malberg *et al*, 2000; Santarelli *et al*, 2003; van Praag *et al*, 1999).

The Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) are used as a genetic animal model of depression and controls, respectively. Selective breeding of Sprague–Dawley (SD) rats with regard to their response to the anticholinesterase agent diisopropylfluorophosphate (DFP)

*Correspondence: Dr AA Mathé, Karolinska University Hospital-Huddinge, SE-141 86 Stockholm, Sweden, Tel: +46 8 524 879 72, Fax: 46 8 524 88929, E-mail: aleksander.mathe@cns.ki.se
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(Overstreet *et al*, 1979; Russell *et al*, 1982) generated a sensitive (FSL) and a resistant strain (FRL). Subsequently, it was realized that FSL exhibits a phenotype that mimics a number of symptoms seen in human depression (Husum *et al*, 2001; Overstreet, 1993; Pucilowski *et al*, 1993). The FSL strain shows good face and predictive validity as a model of depression and in assessment of antidepressant treatments (Overstreet, 1993, 1995). Recently, we found that the antidepressant effect of exercise in FSL was associated with increased hippocampal cell proliferation (Bjørnebekk *et al*, 2005). Thus, increased cell proliferation and neurogenesis in the SGZ appear to be one common feature of antidepressant treatments, including running.

Several different molecules such as trophic factors and neuropeptides are suggested to have an important role in the control of cell proliferation, neurogenesis, differentiation, and cell survival. In the adult olfactory epithelium neuropeptide Y (NPY) has been demonstrated to promote neuronal proliferation (Hansel *et al*, 2001a). Moreover, clinical and experimental evidence suggests a role for NPY in the pathophysiology of depression (Mathé and Gruber, 2004; Nikisch *et al*, 2005; Widerlov *et al*, 1988). Previous clinical studies have reported decreased NPY levels in brain tissue from suicide victims (Widdowson *et al*, 1992) and in the cerebrospinal fluid (Heilig *et al*, 2004) and plasma (Nilsson *et al*, 1996) of depressed patients compared to controls. In rats intracerebroventricular (i.c.v) administration of NPY is anxiolytic (Britton *et al*, 1997; Heilig *et al*, 1989) and decreases immobility in the Porsolt swim test (PST) (Mathé and Gruber, 2004; Stogner and Holmes, 2000). Further, the effects of NPY in the PST are dose dependently blocked by an NPY-Y1 receptor antagonist (Mathé and Gruber, 2004). In genetic animal models of depression, the Fawn Hooded rats and FSL, decreased levels of hippocampal NPY were found (Jiménez Vasquez *et al*, 2000a, b). Furthermore, NPY is also decreased in rat models that mimic environmental stress, such as maternally separated rats (Husum and Mathé, 2002; Jiménez-Vasquez *et al*, 2001; Woldbye *et al*, 2002). Conversely, antidepressant treatments, for example, chronic but not single ECT in humans and electroconvulsive stimuli (ECS) in rodents, lithium and antidepressant drugs (Stenfors *et al*, 1989; Mathé *et al*, 1990, 1994, 1996; Mathé, 1999; Caberlotto *et al*, 1999). Cumulatively, these results indicate that NPY might be involved in affective disorders and their treatments via a mechanism that could include regulation of cell proliferation and neurogenesis in hippocampus. However, it remains unclear if manipulating NPY clinically would have antidepressive and neurogenic effect in depressed individuals (Mathé *et al*, 2005).

The opioid peptides dynorphin and enkephalin are expressed with high levels in dentate gyrus and described to have a putative role in the control of mood (Filliol *et al*, 2000) as well as in regulation of cell proliferation and neurogenesis of adult hippocampal progenitors (Persson *et al*, 2003; Shirayama *et al*, 2004). These endogenous opioid peptides are also highly expressed in the granule cell layer of hippocampus, a brain region involved in memory formation. Virtually all neurons in the granule cell layer express dynorphin and a subset of neurons within this layer also expresses enkephalin.

In hippocampus, dynorphin has an inhibitory effect on long-term potentiation (LTP) (Wagner *et al*, 1993), but less is known about its effects in modifying affective states. In contrast to dynorphin, enkephalin potentiates LTP in hippocampus (Bramham *et al*, 1991). Most likely, enkephalin binds to mu and delta receptors on inhibitory GABAergic interneurons within the CA3 and CA1, thereby facilitating LTP in hippocampus via disinhibition of excitatory pyramidal neurons within CA3 and CA1. Based on results from animal studies, it was suggested that delta receptors have a potential in treatment of depression (Broom *et al*, 2002). Thus, mice with a deletion of delta receptors have increased anxiety and 'depression'. Moreover, delta receptor agonists and enkephalinase inhibitors have an antidepressant-like effect in animal studies (Tejedor-Real *et al*, 1998).

In a previous study we showed that FSL rats have lower basal cell proliferation compared to FRL rats. When placed in single cages with free access to running wheels, the FSL rats develop a lower daily running than the FRL rats. After 5 weeks, the distances covered were approximately 3 and 7 km/day by the FSL and FRL rats, respectively. Running during 30 days had an antidepressant-like effect as tested in the PST and also increased cell proliferation in the FSL but not in the FRL rats (Bjørnebekk *et al*, 2005). In contrast, running increased BDNF levels in the FRL but had no effect in the FSL rats. The aim of this work was to, in the same animal model, study the effects of running on regulation of NPY and the opioid peptides dynorphin and enkephalin in hippocampus and to correlate possible neuropeptide changes with the effects of running on a measure of depression (the PST) and cell proliferation.

MATERIALS AND METHODS

Wheel Running

Male FSL rats ($n = 16$), and their controls, FRL ($n = 16$) were bred at the Karolinska Institute. All animal experiments were approved by the Ethical Committee for Animal Research in Stockholm. The animals were individually housed with either free access (FSL, $n = 8$; FRL, $n = 8$) or no access (FSL, $n = 8$; FRL, $n = 8$) to running wheels (diameter, 34 cm; one revolution corresponding to 1.07 m) during a period of 5 weeks (Figure 1). Running data were sampled

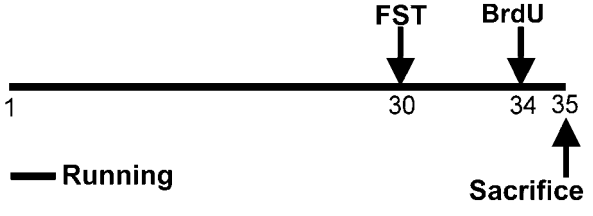

Figure 1 A schematic illustration of the experimental design. Runners ($N = 16$) had free access to the running wheels from experimental day 1–35. Control animals ($N = 16$) went through the same protocol except that they had no access to the running wheels. On day 30, a modified Porsolt swim test was performed on all animals ($n = 32$). On day 34, all animals received four injections of BrdU (75 mg/kg, i.p.) and were killed about 20 h after the last injection.

48 times/day using a computer-based data system with a customized software (Werme *et al*, 1999). Animals had access to food and water *ad libitum* and were subjected to a controlled 12-h light : 12-h dark-schedule (lights on at 0700). After 30 days of running, animals were analyzed in a modified version of Porsolt's swim test (see Bjørnebekk *et al*, 2005).

5-bromo-2deoxyuridine (BrdU) Administration

To evaluate cell proliferation, four injections of BrdU (75 mg/kg, i.p., Sigma) with 2 h intervals were administered 4 days after the swim test (Bjørnebekk *et al*, 2005). One FSL runner and one FSL control died after BrdU injections leaving seven FSL runners and seven FSL controls for histological analysis. The animals were killed about 20 h after the last injection (Figure 1). The brains were immediately removed and frozen at -80°C.

BrdU Immunohistochemistry

For the immunohistochemistry, coronal 40 μm sections were collected with a cryostat throughout the hippocampal formation and temporarily stored in -20°C freezer. Antibodies and dilutions used were: 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 postfixed for 10 min in 4% formaldehyde, rinsed in 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 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, USA), 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 40-μm sections were taken throughout the hippocampus and every 15th section (600-μm apart) (section sampling fraction (ssf)) was selected for analysis of the right dentate gyrus. On average, eight sections per animal were analyzed. An unbiased counting frame with known area was superimposed on the field of view by appropriate software (Stereologer™, SPA Inc.). 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 steps 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

$$N_{\text{total}} = \sum Q^{-} \frac{1}{\text{ssf}} \frac{1}{\text{asf}} \frac{t}{h}$$

where $\sum Q^{-}$ is the number of neurons counted in the dissectors. The dentate gyrus was manually outlined using a $\times 10$ lens. Cell counts were performed with a $\times 60$ 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 uppermost 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 (40-μm) were cut on a cryostat at -20°C, and sections were thawed on glass slides. The hybridization cocktail contained 50% formamide, $4 \times$ SSC ($1 \times$ SSC is, in M, NaCl, 0.15; sodium citrate, 0.015, pH 7.0), $1 \times$ Denhardt's solution, 1% Sarcosyl, 0.02 M Na_3PO_4, pH 7.0, 10% dextransulphate, 0.06 M dithiothreitol, and 0.1 mg/ml sheared salmon sperm DNA. Single-stranded oligonucleotide 48-mer DNA probes specific for dynorphin (296–345) (Douglass *et al*, 1989), enkephalin (235–282) (Zurawski *et al*, 1986), and NPY (1671–1714) (Larhammar *et al*, 1987) mRNA were used. The probes were 3′-end labeled with α-^{33}P-dATP (Dupont NEN, Wilmington, DE) using terminal deoxynucleotidyl transferase (Gibco) to a specific activity of approximately 1×10^9 c.p.m./mg. Hybridization was performed for 18 h in a humidified chamber at 42°C. Following hybridization, the sections were rinsed 4×20 min in $1 \times$ SSC at 60°C and subsequently 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 5–12 days and 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 Analysis

Two-way MANOVA with planned comparison and Scheffe *post hoc* test was performed to analyze mRNA levels. To further investigate the relationship between treatment and outcome variables Persons product-moment correlation was calculated (Statistica).

RESULTS

Basal Levels of NPY, Dynorphin, and Enkephalin mRNAs in Hippocampal Subregions and the Effect of 5 weeks of Running

To analyze the neurochemistry that underlies the adaptive differences between the 'depressed' FSL and the control FRL rats, mRNAs encoding NPY and the opioid neuropeptides dynorphin and enkephalin were analyzed in hippocampal subregions in single housed rats with/without access to running wheels.

Analyses of basal NPY mRNA levels in the two strains revealed similar levels of NPY mRNA in CA1 and CA3. In the dentate gyrus, NPY mRNA was lower in the FSL compared to FRL rats ($p<0.01$). Running had no effect on NPY mRNA in CA1 and CA3 but it increased NPY mRNA in the CA4 and dentate gyrus in the FSL compared to the FRL rats ($p<0.05$) (Figure 2). Baseline NPY mRNA levels did not differ between the dorsal and the ventral blade of the dentate gyrus, and running had no differential effect in dorsal *vs* ventral blade (data not shown). In a recent study we showed that FSL rats have low cell proliferation that can be normalized by running (see Table 1, reviewing cell proliferation data from Bjørnebekk *et al*, 2005). Interestingly, NPY mRNA in the CA4 region and in the dentate gyrus was positively correlated to the number of proliferated BrdU-positive cells in the subgranular zone of dentate gyrus ($r=0.41$, $r=0.52$, $p<0.05$) (Figure 3) (BrdU data from Bjørnebekk *et al*, 2005).

Dynorphin mRNA levels differed between the two strains in the dentate gyrus ($p<0.001$), with lower levels in the FSL rats. In all groups the dynorphin mRNA levels were equally expressed in the dorsal and the ventral blade, and thus not separating the two regions (data not shown). Running decreased dynorphin mRNA in the FRL strain ($p<0.01$) but not in the FSL strain (Figure 4). There was no correlation between cell proliferation and levels of dynorphin mRNA in hippocampus. Enkephalin mRNA levels were lower in the dorsal and in the ventral blade of the dentate gyrus ($p<0.01$) in the FSL compared to FRL rats. There was a trend to an increase in the ventral blade of the dentate gyrus in FRL runners ($p=0.08$) (Figure 5). Levels of enkephalin mRNA in hippocampus were not correlated to cell proliferation.

Pearson's correlations between immobility time in the PST (see Bjørnebekk *et al*, 2005) and the neuropeptides analyzed were not statistically significant for any hippocampal subregion (data not shown).

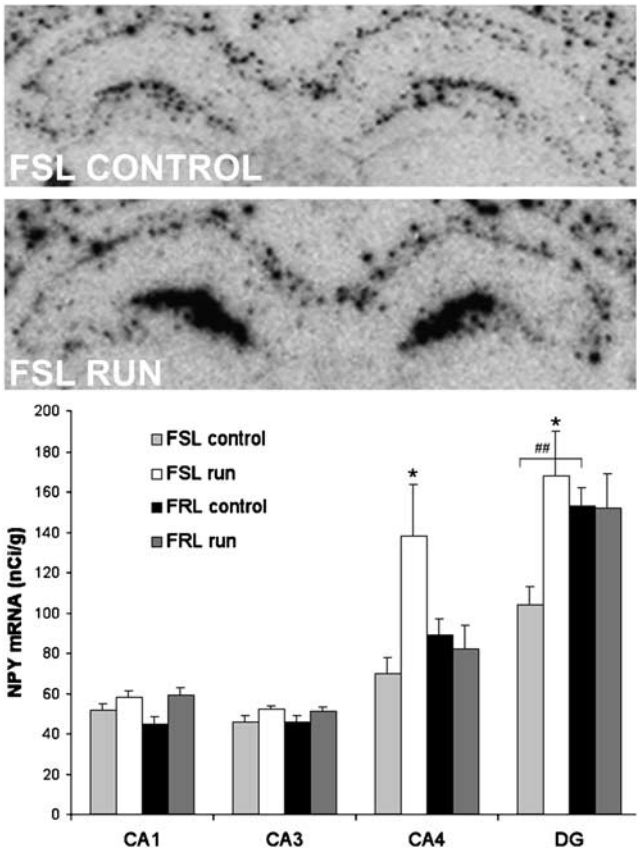

Figure 2 NPY mRNA expression in single housed FRL and FSL rats after 5 weeks of running. Upper panels show an *in situ* autoradiogram of NPY mRNA expression in hippocampus. A 48-mer oligonucleotide probe specific for NPY mRNA was used. Note the increase in NPY mRNA expression in the dentate gyrus and CA4 region of the hippocampus in FSL runners. Lower panel graphs show the levels of hippocampal NPY mRNA in FSL and FRL rats with/without access to running-wheels ($n=6$–8/group). Analyses were performed approximately at the level of Bregma −3.30. Values are means ± SEM. $^{\#\#}p<0.01$ indicates a strain difference, $^{*}p<0.05$ indicates significantly higher levels of NPY mRNA after running. Ca1-4, fields of Ammon's horn; DG, dentate gyrus.

Table 1 Cell Proliferation in the Dentate Gyrus in FRL and FSL Rats with/without Access to Running Wheels

	Control	Run
FRL	100 ± 19.36	116 ± 35.35
FSL	25 ± 1.86	110 ± 14

The FRL controls were arbitrarily denoted as 100%. FSL controls have lower cell proliferation than FRL controls. Running increases cell proliferation in FSL rats to about the same level as FRL controls. For more details on cell proliferation data see Bjørnebekk *et al* (2005).

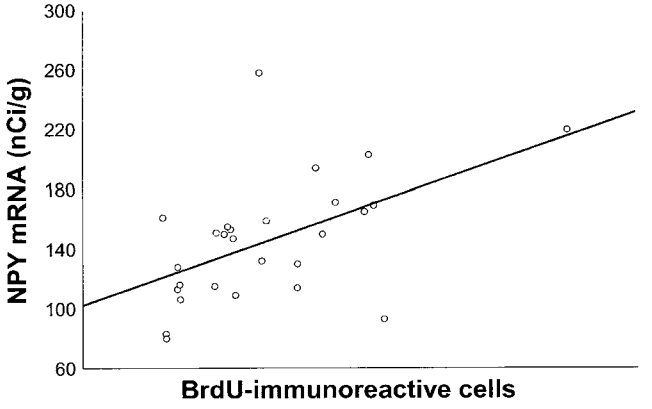

Figure 3 NPY mRNA levels in the dentate gyrus from FSL and FRL rats with/without access to running wheels correlate with the number of newly proliferated cells positive for BrdU-immunoreactivity in the subgranular layer of dentate gyrus. Each dot represents one rat ($n=27$) ($r=0.52$, $p<0.05$).

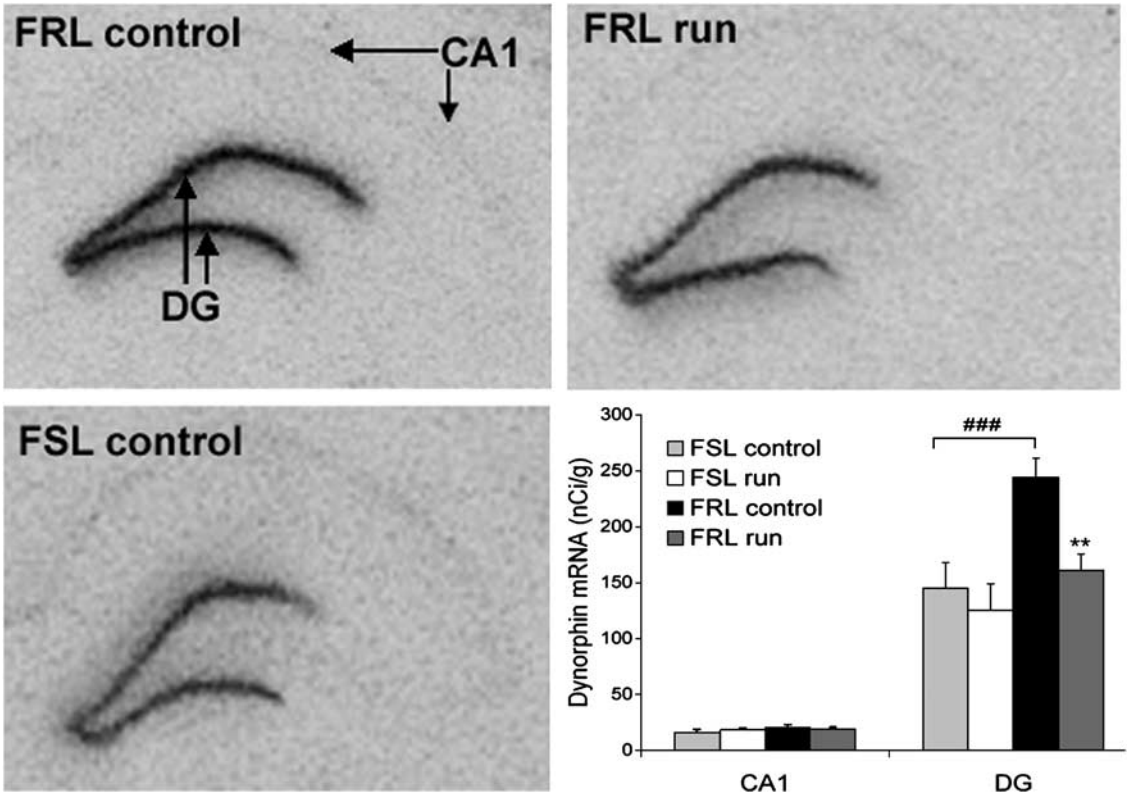

Figure 4 Hippocampal dynorphin mRNA expression in FSL and FRL rats after 5 weeks of running. *In situ* autoradiogram of dynorphin mRNA in the dentate gyrus of FSL and FRL rats and effect of running. Note the lower expression of dynorphin mRNA in FSL and a dynorphin mRNA decrease in the ventral and dorsal blade of the dentate gyrus in FRL rats after running. Bars illustrate dynorphin mRNA levels in hippocampal subregions in FSL and FRL rats with/without access to running wheels ($n = 6$–8/group). FRL controls have a higher baseline level of dynorphin mRNA in the ventral and dorsal blade of the dentate gyrus than the FSL controls. Running decreases dynorphin mRNA levels in the dentate gyrus in FRL rats. Analyses were performed approximately at the level of Bregma −3.30. Values are means ± SEM. $^{**}p < 0.01$ indicates a significant decrease in dynorphin mRNA levels after running. $^{###}p < 0.001$ indicates a strain difference. Ca1, field of Ammon's horn; DG, dentate gyrus.

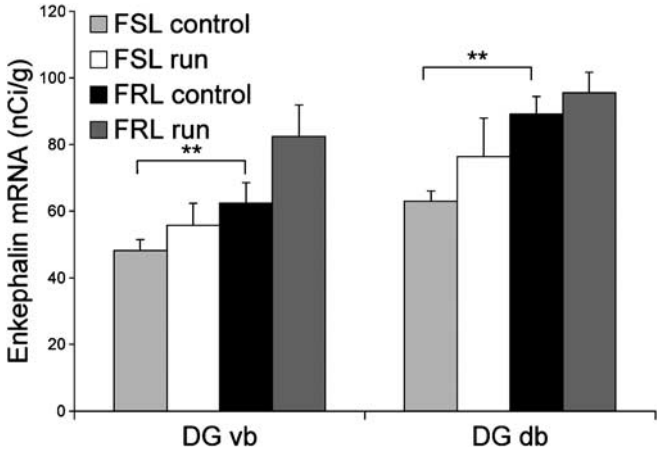

Figure 5 Hippocampal enkephalin mRNA expression in FSL and FRL rats after 5 weeks of running. Bar graphs illustrate enkephalin mRNA levels in the dorsal (DG db) and ventral blade (DG vb) of dentate gyrus in FSL and FRL rats with/without access to running-wheels ($n = 6$–8/group). FRL controls have a higher baseline level of enkephalin mRNA in dorsal and ventral blade of the dentate gyrus than FSL controls. Running did not change enkephalin mRNA levels in the dentate gyrus of FRL and FSL rats. Analyses were performed approximately at the level of Bregma −3.30. Values are means ± SEM. $^{**}p < 0.01$ indicates a strain difference in enkephalin mRNA levels. DGvb, dentate gyrus ventral blade; DGdb, dentate gyrus dorsal blade.

DISCUSSION

Physical activity has an antidepressant effect in humans (Babyak *et al*, 2000; Martinsen, 1990) and an antidepressant-like effect in the 'depressed' FSL rats as assessed in the PST (Bjørnebekk *et al*, 2005). The antidepressant-like effect is associated with increased cell proliferation in the subgranular zone of the dentate gyrus (Bjørnebekk *et al*, 2005). In the present study we further analyzed whether there are differences in basal mRNA levels of NPY and opioid peptides enkephalin and dynorphin between the FSL and FRL rats in hippocampus and if running will alter expression of these neuropeptides in hippocampal subregions.

The Antidepressant-Like Effect of Running in FSL Rats is Associated with Increased NPY mRNA in Hippocampus, which is Correlated to Increased Cell Proliferation

Basal NPY mRNA level in the dentate gyrus was lower in the FSL compared to FRL rats. Following running there was a marked NPY mRNA increase in CA4 and the dentate gyrus in the FSL but not in FRL rats. In the FSL rats, running induced increase in NPY mRNA was strongly correlated to

the running induced increase in cell proliferation (Bjørnebekk *et al*, 2005). Consequently, it is conceivable that NPY is one of the factors that trigger cell proliferation. Consistent with such reasoning is the finding that NPY increases adult neurogenesis in the olfactory bulb (Hansel *et al*, 2001b). Thus, NPY has the potential to increase neurogenesis in the adult brain.

The Y1 receptor, which promotes neurogenesis in olfactory neurons (Hansel *et al*, 2001a), is abundant in the hippocampus and it is possible that its stimulation by running in the dentate gyrus leads to increase in cell proliferation. However, additional studies are needed to ascertain this putative mechanism. Another possible interpretation of our data is that the increase in NPY and cell proliferation seen in FSL rats after running is caused by other variables not measured in this study. In either case, NPY and cell proliferation could be important targets for novel treatment of depression.

Humans studies have demonstrated decreased levels of NPY in the cerebrospinal fluid in depressed patients (Gjerris *et al*, 1992; Widerlov *et al*, 1988) and in postmortem brain tissue from suicide victims (Widdowson *et al*, 1992). However, conflicting results also exist (Berrettini *et al*, 1987). Antidepressants and ECT elevate NPY in human CSF and NPY, both protein and mRNA, in selected rat brain regions, predominantly in the hippocampal formation (Jiménez Vasquez *et al*, 2000a, b; Mathé *et al*, 1996; Nikisch *et al*, 2005). While ECT and ECS consistently increase NPY (Husum *et al*, 2000; Mathé *et al*, 1996; Mikkelsen *et al*, 1994; Stenfors *et al*, 1989; Zachrisson *et al*, 1995), the effects of antidepressants are more inconsistent (Bellmann and Sperk, 1993; Berrettini *et al*, 1987; Heilig *et al*, 1988). In both environmental and genetic animal models of depression, lower levels of NPY mRNA have been found in the CA region (Caberlotto *et al*, 1999; Husum and Mathé, 2002; Husum *et al*, 2002). In the present study, lower levels of NPY mRNA in the dentate gyrus were found in FSL rats, confirming the previous studies. Our data show that also running, which is antidepressant in humans (Babyak *et al*, 2000; Martinsen *et al*, 1989), increases the levels of NPY mRNA in the dentate gyrus and in the CA4 region of hippocampus in the 'depressed' FSL but not in the 'nondepressed' FRL rats. The increase in NPY mRNA does not seem to be dependent on running distance. NPY mRNA levels were not altered in the FRL that showed higher running behavior than the FSL strain. Thus, these two strains exhibit different response to running. These results further demonstrate that the same treatment or behavior can have different effects on NPY and highlight the importance of testing potential antidepressant treatments on depressed animals.

On the molecular level, this selective NPY regulation could be due to transcription factors acting directly on the NPY promoter such as NGFI-A, AP-1, AP-2, and retinoic acid, which might act with different potencies on the promoter of the NPY gene in the two strains (Buckland *et al*, 2004; Jalava and Mai, 1994; Li *et al*, 2000; Magni *et al*, 2000; Wernersson *et al*, 1998). Moreover, it is conceivable that there is a set of factors controlling basal transcription and another set that is induced after running. This might explain the finding that running induced increase in NPY mRNA in 'depressed' but not in 'nondepressed' animals.

Antidepressant-Like Effect of Running in FSL Rats is not Dependent on Regulation of Dynorphin and Enkephalin mRNAs

Dynorphin, another neuropeptide expressed in the dentate gyrus and striatum, is also involved in regulation of mood. Dynorphin agonists are dysphoric in humans (Pfeiffer *et al*, 1986). In the nucleus accumbens, kappa receptor agonists reduce dopamine release (Spanagel *et al*, 1992), whereas they inhibit excitatory glutamatergic neurotransmission in the hippocampus (Wagner *et al*, 1993). Recently, we demonstrated that running increases dynorphin mRNA in the dorsal striatum in FRL rats and in nucleus accumbens in both FRL and FSL rats (Bjørnebekk *et al*, 2005). In the present study we analyzed the effects of running in the two strains on dynorphin mRNA in hippocampus. Interestingly, basal dynorphin levels were higher in the dentate gyrus of FRL than FSL rats. After running, dynorphin mRNA was decreased in the FRL but not in the FSL strain. In hippocampus, dynorphinergic kappa receptors are localized on presynaptic terminals of the entorhinal cortex and also in the pyramidal cell layer (Mansour *et al*, 1988). In dorsal and ventral striatum kappa receptors are localized on presynaptic mesostriatal and mesolimbic nerve terminals. It is conceivable that the reduced hippocampal dynorphin mRNA after running in the FRL rats and the increased dynorphin mRNA in dorsal and ventral striatum reflect a functional adaptation in the limbic system at a network level. Thus in FRL rats, decreased dynorphin in hippocampus most likely reflects facilitation of hippocampal activity, which ultimately could potentiate learning and memory but has no effect on depression. On the other hand, increased dynorphin in ventral striatum implies that dynorphin mediates an inhibitory tone in the mesostriatal mesolimbic system. This inhibitory tone on the mesolimbic reward system could serve as a brake to inhibit the rats to develop a compulsive running behavior. The transcription factor CREB controls transcription of the preprodynorphin gene (Cole *et al*, 1995). Genetically engineered animals with increased levels of CREB in the nucleus accumbens also show increased immobility in the swim test. This can be counteracted by local administration of a kappa receptor antagonist, suggesting that the high immobility in the swim test in rodents with high CREB levels in nucleus accumbens is caused by an interaction of dynorphin and its receptor in nucleus accumbens (Pliakas *et al*, 2001). In hippocampus, the levels of dynorphin are increased after immobilization stress and after training in the learned helplessness paradigm. In this model, infusions of a kappa receptor antagonist into both nucleus accumbens and hippocampus result in improvement in the conditioned avoidance test (Shirayama *et al*, 2004). In our study, baseline level of dynorphin mRNA expression in the dentate gyrus was higher in the FRL compared to the FSL rats. After running, dynorphin mRNA levels were decreased in the dentate gyrus in FRL rats but not in the 'depressed' FSL rats. Therefore, we conclude that dynorphin mRNA regulation after running is not an important factor for the antidepressant-like effect of running or for increased cell proliferation in the FSL strain. On the other hand, and independently of depression, it is conceivable that there is a link between the amount of running and the degree of downregulation of dynorphin

mRNA in the dentate gyrus. Kappa receptor activation will generate decreased cAMP levels, and thus downregulation of dynorphin could lead to increased activity and increased cAMP levels in cells expressing kappa receptors. In fact, in a previous study, we demonstrated that BDNF mRNA levels in the dentate gyrus were increased after running in FRL but not in FSL rats. One putative mechanism is that running downregulates kappa receptor activity leading to increased intracellular cAMP levels and increased BDNF transcription in hippocampus. In contrast to dynorphin, enkephalin potentiates LTP in hippocampus (Bramham *et al*, 1991). Most likely, enkephalin binds to mu and delta receptors on inhibitory GABAergic interneurons within the CA3 and CA1, thereby facilitating LTP in hippocampus via disinhibition of excitatory pyramidal neurons within CA3 and CA1. Broom *et al* (2002) reported that SCN80, a delta receptor agonist, increases climbing behavior but does not affect swimming in the PST. Since climbing activity primarily reflects the dopaminergic activity (Reneric and Lucki, 1998), these results probably indicate an interaction between the doapminergic and opioid system in the rewarding pathways.

In this context it is of interest that mice with a deletion of delta receptors show increased anxiety and 'depression' (Filliol *et al*, 2000). Moreover, delta receptor agonists and enkephalinase inhibitors modify the escape behavior in the learned helplessness model although at high doses they are also epileptogenic (Tejedor-Real *et al*, 1998). Under basal conditions, enkephalin mRNA expression in dentate gyrus is higher in the FRL than in the FSL rats. However, in contrast to dynorphin, enkephalin mRNA was not changed after running. Our results thus do not indicate that enkephalin is involved in regulating the increase in cell proliferation after running or that it plays a role in the antidepressant-like effect of running. However, they do raise the possibility that in the FSL strain, the low basal levels of enkephalin in hippocampus could be a factor contributing to the low cell proliferation and the state of depressive-like phenotype.

Conclusion

Accumulated evidence indicates that a dysregulation of the NPY system plays a role in pathophysiology of depression and that one mechanism of action shared by antidepressive treatments, pharmacological as well a physical, is enhancement of the NPYergic transmission (Mathé, 1999; Mathé *et al*, 1997, 1998, 1996, 2005). The increase of hippocampal NPY mRNA following wheel running found in this study is in line with this hypothesis and further extends it by demonstrating that physical activity that has antidepressive effects also enhances NPY expression in hippocampus. In addition, the demonstrated strong correlation between running induced cell proliferation and NPY mRNA suggests that increase in cell proliferation after antidepressive treatments could be stimulated by NPY.

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