

Antidepressant actions of the exercise-regulated gene VGF

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Exercise has many health benefits, including antidepressant actions in depressed human subjects, but the mechanisms underlying these effects have not been elucidated. We used a custom microarray to identify a previously undescribed profile of exercise-regulated genes in the mouse hippocampus, a brain region implicated in mood and antidepressant response. Pathway analysis of the regulated genes shows that exercise upregulates a neurotrophic factor signaling cascade that has been implicated in the actions of antidepressants. One of the most highly regulated target genes of exercise and of the growth factor pathway is the gene encoding the VGF nerve growth factor, a peptide precursor previously shown to influence synaptic plasticity and metabolism. We show that administration of a synthetic VGF-derived peptide produces a robust antidepressant response in mice and, conversely, that mutation of VGF in mice produces the opposite effects. The results suggest a new role for VGF and identify VGF signaling as a potential therapeutic target for antidepressant drug development.

Exercise has well known cardiovascular¹ benefits; however, it has only recently been documented that exercise also augments brain function and mental health. For instance, exercise enhances hippocampal learning and improves executive functioning in aging humans, while also providing protection from brain insults and disease^{2–5}. In addition, recent studies have shown that exercise produces antidepressant responses in rodent models⁶ and mood-elevating actions in humans^{7,8}. The mechanisms underlying the beneficial effects of exercise are not fully understood and could be targets for new therapies that are different from traditional chemical antidepressants.

The antidepressant actions of exercise are particularly noteworthy because of the prevalence of depression (16% in the US population)⁹, its enormous economic burden (\$83.1 billion per year in the US)¹⁰ and a tremendous need for more effective treatments. Current antidepressant medications are effective for approximately 65% of depressed patients and require long-term treatment for weeks to months before a therapeutic response is achieved¹¹.

The time lag in the therapeutic response, in spite of the rapid (hours to days) increase in monoamine levels, has led to the hypothesis that neural adaptations or plasticity are required. One of the adaptations that may contribute to the actions of antidepressants is the upregulation of neurotrophic factors, most notably brain-derived neurotrophic factor (BDNF), in limbic structures that have been implicated in depression¹². Notably, exercise also increases the expression of BDNF.

Here we extend these results by using a focused microarray to show that exercise upregulates a primary signaling cascade for neurotrophic factors and a peptide precursor, VGF, that has strong antidepressant efficacy in behavioral models.

RESULTS

Exercise gene profile

To profile hippocampally expressed, exercise-regulated genes, we have developed a custom microarray optimized for analysis of the relatively small changes in gene expression that are typically observed in heterogeneous tissue such as brain¹³. We have concentrated on the hippocampus, a limbic structure that is highly sensitive to stress hormones, shows metabolic and morphological alterations in the brains of depressed subjects and mediates antidepressant responsiveness in behavioral models of depression¹⁴. In addition, the hippocampus shows molecular and cellular alterations in response to exercise, including increased expression of neurotrophic factors and adult neurogenesis¹⁵.

We used an exercise model in which mice are individually housed with or without free access to running wheels. By the end of the first week, the mice with wheel access were running approximately 10 km per night (Fig. 1a), and they continued running at this rate for up to 4 weeks (not shown), similarly to other reports in mice¹⁶. Future studies will be required to determine the effects of different amounts of exercise to compare to those in humans. In our study, the mice reached a running plateau more quickly than rats, as was previously reported⁶. For the microarray analysis, we analyzed mice after 7 d of running, the earliest time point at which behavioral effects are observed (data not shown), to capture the early gene expression changes that may underlie the antidepressant response, rather than those observable at a later time point when compensatory gene changes may take place.

Four independent array analyses were performed, each comparing a different pair of sedentary versus exercised mice. The data were subjected to statistical analysis by an unpaired *t*-test using the

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Received 30 May; accepted 20 September; published online 2 December 2007; doi:10.1038/nm1669

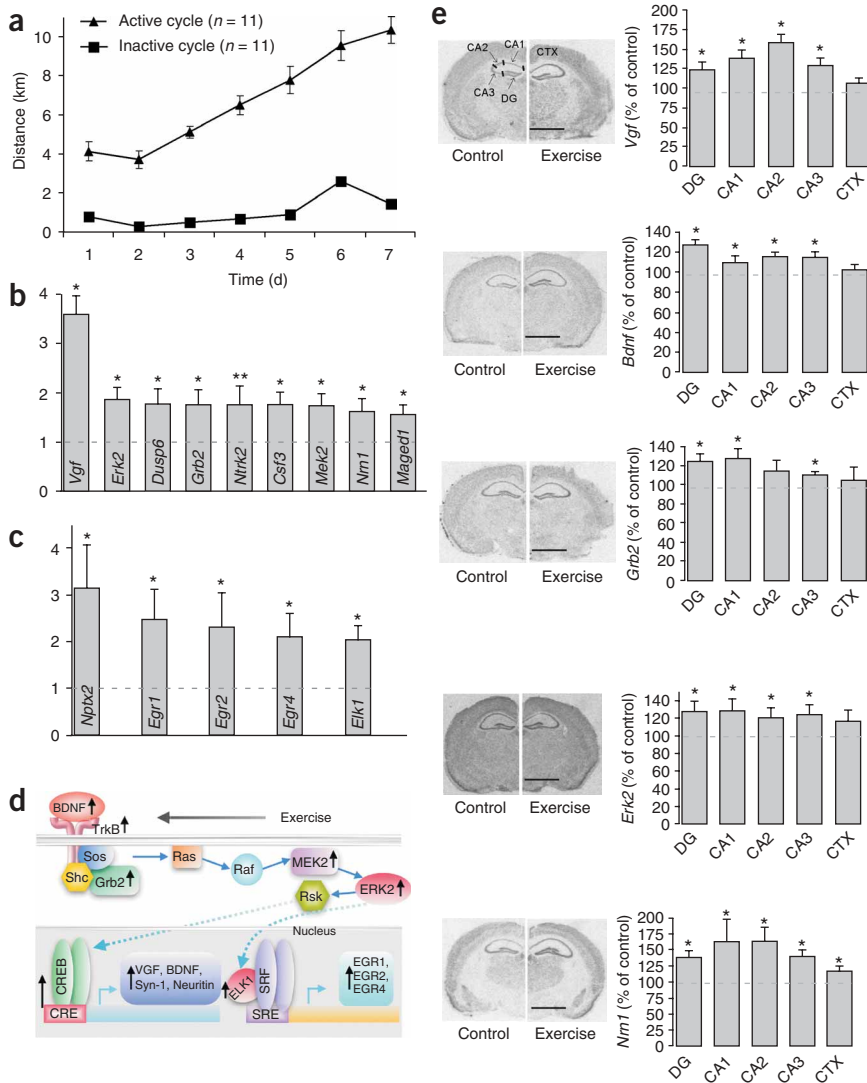


Figure 1 Profile and secondary validation of exercise regulated genes. **(a)** Plot of daily running profile for C57BL/6J mice during active and inactive cycles over 7 d. **(b,c)** Profile of 7-d hippocampal exercise-regulated genes grouped into growth factor **(b)** and transcription factor and immediate early genes **(c)**, as identified by microarray analysis. *Dusp6*, dual-specificity phosphatase-6; *Csf3*, colony-stimulating factor-3; *Maged1*, melanoma antigen, family D, 1; *Nptx2*, neuronal pentraxin-2. **(d)** Diagram of neurotrophic factor signaling pathway and target genes of this cascade showing gene products that are upregulated by exercise (black arrows). **(e)** Secondary confirmation, by *in situ* hybridization, of exercise-regulated genes, including *Vgf* ($n = 15$; top), *Bdnf* ($n = 10$; top middle), *Grb2* ($n = 5$; middle), *Erk2* ($n = 5$; bottom middle) and *Nrn1* ($n = 10$; bottom); * $P < 0.05$ compared to sedentary controls (Student's *t*-test). DG, dentate gyrus; CA1–CA3, Ammon's horn hippocampal subregions; CTX, cortex. Scale bar, 2 mm. * $P < 0.05$; ** $P = 0.06$.

the expression of *Bdnf* was increased by exercise (**Fig. 1e**, top middle), which is consistent with previous reports^{18,19}.

Stimulation of the MAPK cascade also led to increased expression of several gene targets that are related to neurotrophic factor or growth factor function, including VGF, a secreted neuropeptide precursor involved in energy balance²⁰, and neuritin, an immediate early gene involved in neuroplasticity^{21,22} (**Fig. 1b**). The observed induction of VGF is also consistent with observations in a previous microarray study of gene expression changes due to exercise²³. Expression of VGF, as well as of BDNF and neuritin, can be induced by transcription factors that are regulated by the MAPK cascade, including the cAMP response

cross-gene pooled error method. We identified 33 hippocampal exercise-regulated genes (**Supplementary Tables 1 and 2** online), 27 of which had not been previously reported. We categorized these genes into groups on the basis of their function: growth factor signaling genes, transcription factors and immediate early genes, neurotransmitter and synaptic signaling genes, and kinases, phosphatases and enzymes (**Fig. 1b–d** and **Supplementary Tables 1 and 2**). The reproducibility and biological validity of the microarray data are demonstrated by the low standard errors and *P* values (**Supplementary Table 1**) and the high success rate of secondary validation on an independent set of animals (**Fig. 1e**, top).

Many of the identified genes are involved in neurotrophic factor or growth factor signaling. This is noteworthy because of the reported role of neurotrophic factors in the pathophysiology and treatment of depression¹⁷. Several genes that make up the growth factor-stimulated mitogen-activated protein kinase (MAPK) signaling pathway were induced by exercise (**Fig. 1d**), including the genes for neurotrophic tyrosine receptor kinase type-2 (*Ntrk2*), the receptor for BDNF, the adaptor protein growth factor receptor bound-2 (*Grb2*), and two major kinases in the MAPK pathway, mitogen-activated protein kinase kinase-2 (*Map2k2*, denoted here as *Mek2*) and extracellular signal-regulated kinase-2 (*Mapk1*, denoted here as *Erk2*). We also found that

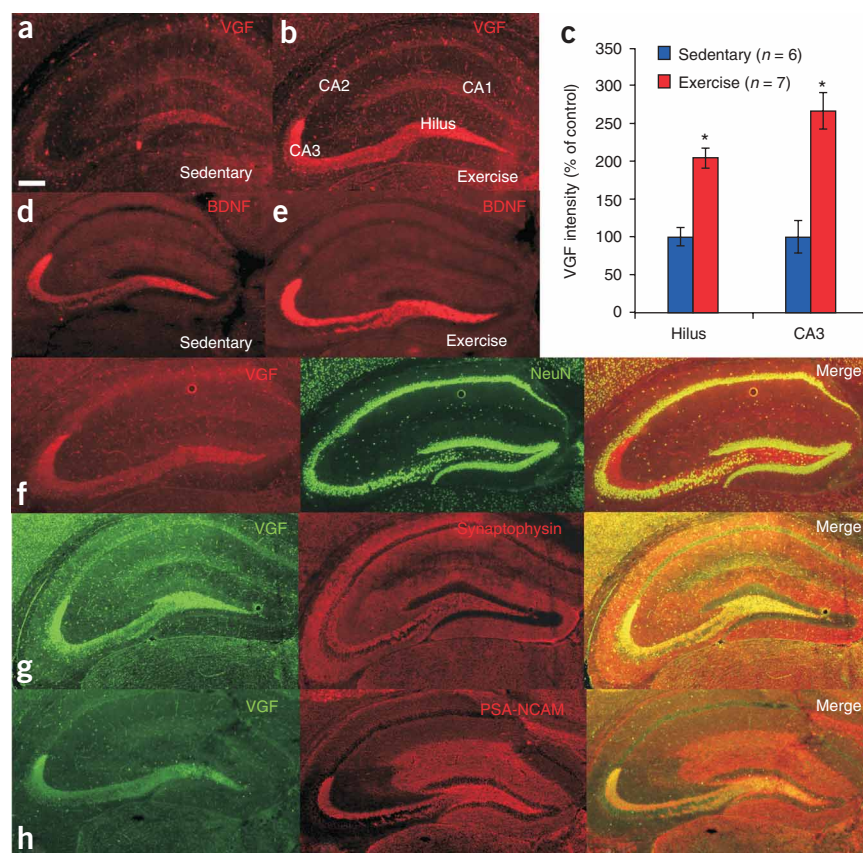
element-binding protein (CREB) or early growth response-1 (EGR1)²⁰. *Egr1*, as well as *Egr2* and *Egr4*, are also upregulated by exercise, and their expression can be induced by another MAPK-regulated transcription factor, *Elk1*, which was upregulated as well (**Fig. 1c,d**).

In situ hybridization analysis of four of these neurotrophic factor- or growth factor-signaling targets was conducted on independent groups of sedentary control and running mice (**Fig. 1e**). The results confirm the microarray data, showing increased expression of *Vgf*, *Grb2*, *Erk2* and neuritin (*Nrn1*) after 1 week of exercise, and they further show the regional localization of these changes in the hippocampus. In most cases, expression was increased in hippocampal subregions, including the dentate gyrus granule cell layer and the CA1, CA2 and CA3 pyramidal cell layers. For comparison, we also examined the expression of these genes in the cerebral cortex, and only neuritin showed significant changes in this region (**Fig. 1e**, bottom). The induction of *Vgf* was also confirmed by RT-PCR ($39.3\% \pm 15.7\%$ increase relative to sedentary controls, mean \pm s.e.m.).

Exercise induces hippocampal VGF protein expression

The robust induction of VGF by exercise, neurotrophic factor signaling and electroconvulsive seizures (ECS), the most efficacious

Figure 2 Analysis of VGF and BDNF immunoreactivity and colocalization with selective neuronal markers after 1 week of exercise. (a,b) Fluorescence immunohistochemistry shows that exercise (7 d) increases VGF expression in the hippocampus. (c) Levels of VGF immunoreactivity were subjected to semi-quantitative analysis of converted grayscale images with US NIH image software. $*P < 0.05$. (d,e) Fluorescence immunohistochemistry shows that exercise (7 d) increases BDNF expression in the hippocampus. (f–h) The cellular localization of VGF after exercise was characterized by colocalization studies with markers of neurons (NeuN, f), synapses (synaptophysin, g) or synaptic remodeling (PSA-NCAM, h). The left column shows VGF, the middle column shows the second marker and the right column shows the merged image. Scale bar, 250 μm (a,b,d–h).



treatment for depression^{23–26}, and the role of VGF in energy metabolism²⁷ and synaptic plasticity²⁸ make it an attractive candidate for mediating the antidepressant actions of exercise. Further analysis of VGF immunoreactivity shows relatively low VGF abundance in the hippocampus of sedentary control mice (Fig. 2a), but noticeable induction of expression in the hilus and CA3 subfields of the hippocampus of exercise mice (Fig. 2b,c). Immunohistochemistry shows a similar pattern of BDNF expression (Figs. 2d,e), suggesting that exercise-induced hippocampal regulation of both VGF and BDNF may occur through similar signaling pathways. In support of this possibility, we have also found that stress decreases both *Vgf* and *Bdnf* mRNA abundance in the dentate gyrus (Supplementary Fig. 1 online). Colocalization studies with markers for neurons (NeuN), synapses (synaptophysin) and synaptic remodeling (PSA-NCAM) showed that VGF protein is expressed primarily in axons of the mossy fiber pathway and synaptic terminals in the CA3 pyramidal cell layer (Figs. 2f–h), indicating that most of the VGF is transported out of the granule cells.

VGF produces an antidepressant-like effect in mice and rats

To test directly whether VGF could contribute to antidepressant actions, we determined the influence of synthetic VGF on behavioral models of depression and antidepressant responsiveness. A VGF peptide (AQEE-30 amino acids 588–617) previously shown to increase hippocampal synaptic charge²⁸ was microinfused into the lateral ventricles of mice. Their behavior in the forced-swim or tail-suspension tests, two widely used animal models of antidepressant responsiveness²⁹, was monitored. Although it is difficult to model complex behaviors such as depression in rodents, most classes of antidepressants increase escape-directed behaviors or decrease immobility in these models with a potency that correlates with clinical efficacy in humans²⁹. VGF infusions significantly decreased immobility times in both of these tests (Fig. 3a,b).

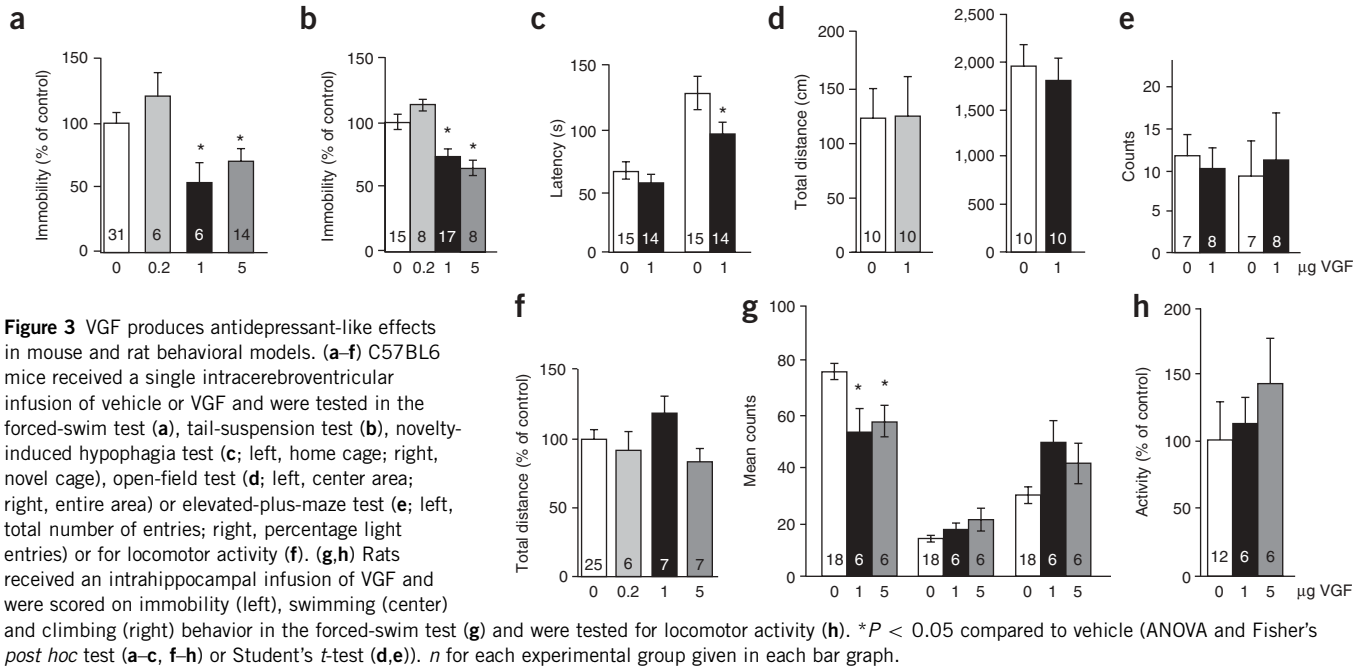
VGF infusions were also tested in the novelty-induced hypophagia model, in which the mouse is sensitive to chronic, but not acute, chemical antidepressant treatment³⁰. This model uses an anxiety-related measure (hyponeophagia, or inhibition of feeding in a novel environment) that differs from the forced-swim test and

tail-suspension test. VGF treatment significantly reduces novel-cage latency in the novelty-induced hypophagia model (Fig. 3c) but did not influence home-cage latency to feed. These results provide further evidence to support VGF's antidepressant actions. Infusions of VGF did not influence locomotor activity or behavior in two models of anxiety in mice, the elevated-plus-maze model or the open-field activity model (Fig. 3d–f and Supplementary Table 3 online). The open-field test provides an additional measure of locomotor activity (total distance traveled) and further shows that VGF does not have a general effect on ambulatory activity.

We further investigated the actions of VGF in the rat to evaluate hippocampal specificity (bilateral infusions into the hippocampus) and behaviors that are suggestive of the activity of serotonin (swimming) or norepinephrine (climbing) systems²⁹. Local hippocampal infusions of VGF (into the dentate gyrus) significantly decreased immobility (Fig. 3g). The hippocampal infusions of VGF did not significantly increase climbing, swimming or locomotor behavior in rats, although there was a trend toward an increase in swimming (Fig. 3g,h). These results suggest that the effects observed in response to lateral-ventricle infusions in mice could occur, at least in part, via actions on the hippocampus, and they also show that VGF has antidepressant-like effects in two species.

Characterization of heterozygous *Vgf*^{+/-} null mice

We further examined the role of VGF in depressive-like behaviors by testing *Vgf*-null mice. Heterozygous *Vgf*^{+/-} null mice were used because homozygous *Vgf*^{-/-} null mice are lean, hypermetabolic and have lower survival rates²⁷. Notably, we found a very robust deficit, measured by increased immobility, in the sedentary *Vgf*^{+/-} mice in



both the forced-swim test and tail-suspension tests compared with their sedentary wild-type littermates (Fig. 4a,b). These mice have no overt behavioral or physiological abnormalities²⁷ and have normal rates of locomotor activity (Fig. 4c) as well as normal anxiety responses in the elevated-plus-maze and open-field tests (Supplementary Tables 4 and 5 online).

Next we examined the influence of exercise on behavior in the *Vgf*^{+/-} mice. Sedentary *Vgf*^{+/-} mice had immobility deficits in the forced-swim test compared to their wild-type littermates, confirming that immobility is increased in a different cohort of mice. The immobility deficit was also observed in exercised *Vgf*^{+/-} mice, whereas the exercised wild-type mice had an antidepressant-like response (Fig. 4d). *Vgf*^{+/-} mice had lower amounts of VGF in the hippocampus (Fig. 4e), as predicted, consistently with previous reports²⁷. Exercise did increase VGF abundance in the *Vgf*^{+/-} mice, but not to the levels observed in exercised wild-type mice (Fig. 4e). There was no significant difference in locomotor activity between the sedentary and exercised groups across the tested genotypes (Fig. 4f).

Exercise and VGF co-regulate synaptic plasticity genes

We examined the molecular mechanisms underlying the actions of VGF by comparing the VGF- and exercise-induced gene expression profiles. For this assessment, we used a cultured cell line system that allows for control of the dose and duration of VGF exposure. PC12 cells were incubated with the VGF-derived peptide AQEE30 or vehicle and gene expression was examined with a custom cDNA array (see Methods). Notably, we found several synaptic plasticity- and/or neuronal protection-related genes that were regulated by both exercise and VGF (Table 1). These included *Egr2*, *Grb2*, ornithine decarboxylase-1 (*Odc1*), synapsin-1 (*Syn1*) and synCAM (*Igsf4*). Some of these gene products, *Grb2* and *EGR2*, are also in the growth factor signaling pathway (Fig. 1d).

Next we performed *in vivo* VGF infusion experiments with the same paradigm as that used for the behavioral studies. VGF peptide was infused directly into the hippocampal dentate gyrus and expression was analyzed by *in situ* hybridization (Fig. 5). The results show that VGF treatment increases the expression of *Egr2*, *Grb2*, *Nrn1*, *Odc1* and *Syn1*, confirming the exercise and cell culture microarray data for

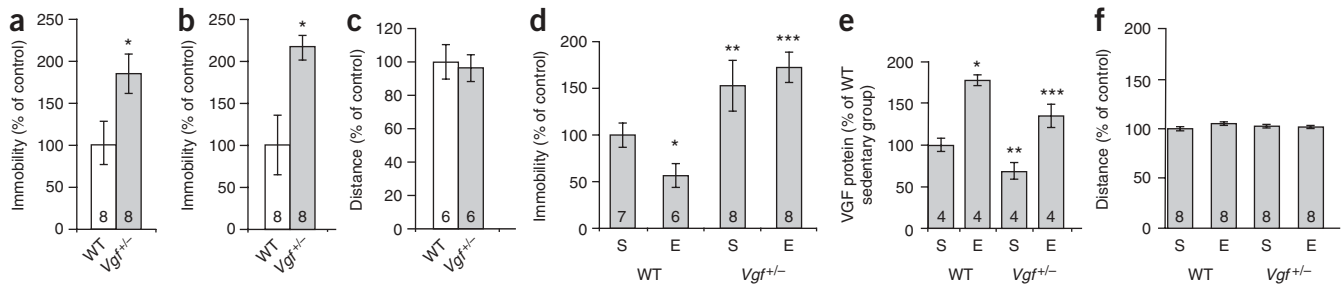


Table 1 Genes co-regulated by both exercise and VGF

	VGF-treated PC12 cells	Exercise (hipp.)	VGF infusions (hipp.)
	Fold regulation	Fold regulation	Fold regulation
<i>Egr2</i>	2.21 ± 0.80 ^a	2.32 ± 0.72 ^a	1.38 ± 0.22 ^a
<i>Grb2</i>	1.78 ± 0.41 ^a	1.75 ± 0.30 ^a	1.49 ± 0.16 ^a
<i>Nrn1</i>	1.86 ± 0.31 ^a	1.61 ± 0.26 ^a	1.30 ± 0.03 ^a
<i>Odc1</i>	1.92 ± 0.31 ^a	1.72 ± 0.34 ^a	1.32 ± 0.08 ^a
<i>Syn1</i>	2.93 ± 0.71 ^a	1.47 ± 0.21 ^a	1.20 ± 0.08 ^a
<i>Cadm1</i>	2.40 ± 0.67 ^a	2.05 ± 0.41 ^a	1.11 ± 0.11 ^b

Genes that are induced by exercise and by VGF in PC12 cells or in response to *in vivo* infusions of VGF into the hippocampus are shown. Gene expression was determined by RT-PCR of PC12 cell extracts, by exercise hippocampal array or by *in situ* hybridization of coronal brain sections after VGF infusions. *P* values (Student's *t*-test) and number of replications are depicted. The SynCAM gene has different names in the rat and in the mouse; the mouse gene name (*Cadm1*) is used in the table for simplicity. Hipp., hippocampal infusion.

^a*n* = 3–4; *P* < 0.05. ^b*n* = 4; *P* = 0.23.

these genes. VGF infusions into the mouse brain (into lateral ventricle) increased the expression of *Grb2*, *Odc1*, *Syn1* and synCAM (*Cadm1* in mice; **Supplementary Fig. 2**). Taken together, the cell culture and *in vivo* infusion experiments provide evidence that the exercise induction of several of these genes occurs through VGF.

DISCUSSION

Collectively, our results show that exercise regulates the expression of *Vgf* mRNA and VGF protein in the rodent hippocampus, that VGF induces a robust antidepressant response in behavioral models and that the opposite phenotype is observed in *Vgf*^{+/-} mice. The upregulation of neurotrophic factor signaling in the hippocampus, as identified by microarray and secondary validation approaches, is consistent with VGF being a downstream gene target of this pathway³¹. These findings further show that exercise facilitates the mechanisms underlying neuroprotection and synaptic plasticity^{32,33} as well as antidepressant responses.

The VGF infusion studies show that VGF can produce antidepressant-like effects in behavioral models, and, conversely, that a deficit in escape-directed behaviors is observed in *Vgf*^{+/-} null mice compared to littermate controls. These data indicate that VGF is sufficient to produce an antidepressant response and that endogenous VGF is required for normal behavior in these antidepressant models. Furthermore, the effects of exercise in the forced-swim test are blocked in the *Vgf*^{+/-} mice, indicating that VGF is required for the antidepressant effects of exercise. These studies provide strong support for the hypothesis that VGF is a novel antidepressant in these rodent models, but analysis of other paradigms that model the core symptoms of depression, such as anhedonia, will be needed to further test the role of VGF in both the pathophysiology and the treatment of depression. In contrast to these results, the behavior of the mice in models of anxiety, including the elevated-plus-maze and open-field tests, was not substantially altered by VGF infusions or in *Vgf*^{+/-} mice, indicating that the antidepressant actions of VGF were relatively selective, although future experiments are still required to fully test this selectivity.

The mechanisms underlying the antidepressant actions of VGF could involve synaptic plasticity and neuroprotection, as stress and depression oppose the mechanisms underlying these effects in the hippocampus, and these mechanisms have been implicated in the etiology of depression^{17,34}. VGF is regulated by hippocampus-dependent learning, and VGF increases hippocampal synaptic charge²⁸. Furthermore, cell culture and *in vivo* infusion studies have

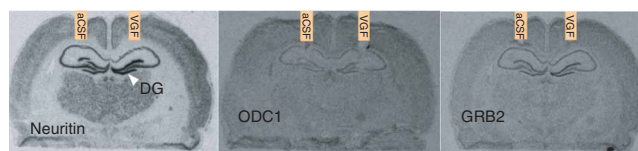


Figure 5 VGF hippocampal infusions induce local gene expression of exercise-regulated genes. Hippocampal infusions of either VGF (right) or artificial cerebral spinal fluid control (aCSF, left) were performed. Depicted are representative autoradiograms of *in situ* hybridization analysis for neuritin, ODC1 and GRB2 performed on hippocampal-infused tissue.

shown that VGF induces synaptic plasticity genes that are also induced after exercise (that is, *Nrn1* and *Syn1*)^{22,35}. The growth factor-stimulated MAPK cascade and at least one of the VGF- and exercise-regulated genes (*Odc1*) also have neuroprotective effects³⁶, and preliminary studies show that VGF protects spinal cord neurons from glutamate-induced toxicity (S.R.S., unpublished data). Promoter analysis shows that many of the genes upregulated by VGF and exercise have CREB and/or EGR1 regulatory elements, suggesting that these two transcription factors are crucial in regulating genes responsive to exercise and VGF (**Supplementary Table 6** online).

It is also noteworthy that ECS, the most efficacious treatment for depression, also increases the expression of several of the exercise- and VGF-induced gene products, including neuritin, EGR2 (ref. 26) and *Grb2* (ref. 37), as well as of VGF itself. This raises the possibility that the therapeutic efficacy of ECS could be mediated in part by the induction of VGF and of one or more of these downstream target proteins. Although there is overlap in the genes regulated by exercise and by ECS, there are also many genes that are differentially regulated by the two, as well as differences in the magnitude of gene expression changes; both distinctions could underlie the greater therapeutic efficacy of ECS. In contrast to ECS, different chemical classes of antidepressants tested to date, including selective serotonin and norepinephrine reuptake inhibitors, do not increase VGF expression (R.S.D., unpublished data), suggesting that there are different therapeutic mechanisms for these agents. However, it is possible that VGF could act either directly or indirectly via the same intracellular signaling pathways as chemical antidepressant agents (for example, CREB (ref. 17)), a possibility supported by promoter analysis of the VGF-regulated genes.

Another possibility is that the actions of VGF are mediated by regulation of energy metabolism. Previous studies have shown that *Vgf*^{-/-} knockout mice are lean and hypermetabolic and that hypothalamic VGF expression is regulated by feeding and fasting^{20,31}. Neuroanatomic pathways between the hippocampus and hypothalamus have been suggested to help regulate ingestive behavior and allow metabolic, emotional and cognitive information to be integrated, thus providing a potential link between observed impairments in appetite and neuropsychiatric conditions, such as depression³⁸. Neuropeptides, including VGF and neuropeptide Y, may therefore modulate both hippocampal and hypothalamic synaptic plasticity, thus coordinating energy balance and behavior and integrating these with external stimuli such as exercise, which has also been reported to enhance hippocampal plasticity³⁹.

A role for neurotrophic factor signaling and its downstream gene targets in the antidepressant actions of exercise is consistent with a large body of literature showing opposing actions of this pathway in the pathophysiology versus the treatment of depression⁴⁰. The selective induction of VGF by exercise, as well as by ECS, also indicates that there are differences in the molecular mechanisms underlying these

treatments in comparison to chemical antidepressants. This highlights the potential importance of VGF as a new endogenous, exercise-regulated target for drug development that could have complementary and possibly even superior efficacy to chemical antidepressants.

METHODS

Animals. Male C57BL/6J mice (weighing 21–26 g, Jackson Laboratory) were provided to us at 6–7 weeks of age (see Acknowledgments). We used adult male Sprague-Dawley rats (260–330 g, Charles River Laboratories) for studies of the behavioral actions of intracerebral infusions and immobilization stress. We housed all animals under standard lighting parameters and gave them food and water *ad libitum*. Animal use procedures were in accordance with the US National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Animal Care and Use Committee.

Custom growth-factor chip. With the assistance of the Keck microarray facility at Yale, we produced a custom cDNA expression array encoding neurotrophic factors, growth factors and related signal transduction genes, as well as transcription factors, G protein-coupled receptors, cAMP response element-regulated genes, and relevant neuropsychiatry-regulated genes. The spotted genes are ~300-bp PCR products. For a full description of the custom neurotrophic factor and growth factor microarray, please see ref. 26.

Microarray analysis of gene expression. We isolated total hippocampal RNA from individual mice (RNA Aqueous, Ambion), reverse-transcribed it into cDNA and then indirectly labeled it with a sensitive fluorescent labeling procedure (Genisphere). We used a two-step hybridization and labeling protocol in which the chip was hybridized to cDNA overnight, washed stringently to remove nonspecifically bound probe and then poststained with fluorescent dendrimers. After the hybridization and washes, we scanned slides with a GenePix scanner (Axon Instruments). We performed image analysis was performed with GenePix Pro 4.0 software (Axon Instruments). The resulting files were imported into Genespring 5.0 (Silicon Genetics) for additional visualization and data mining (see **Supplementary Methods** online for details).

VGF-treated PC12 cell conditions. We examined the VGF responsiveness of undifferentiated PC12 cells by plating PC12 cells on collagen IV-coated six-well dishes, treating them 3 h later with either VGF C-terminal peptide (AQEE; 1 µg/ml) or vehicle (HBSS alone) and incubating them for 6 h. We then washed, solubilized with RNAaqueous (Ambion), sonicated and froze the cells at –80 °C until microarray analysis.

In situ hybridization. We conducted *in situ* hybridization according to standard procedures used in this lab⁴¹.

Immunohistochemistry. Fresh, frozen, cryostat-cut coronal brain sections (14 µm wide) were fixed, incubated in blocking solution (2.5% BSA in PBS), rinsed in PBS and then incubated overnight at 4 °C in primary antibody solution (0.1% Triton-X 100, 1% BSA). We washed sections in PBS and incubated them with fluorescent secondary antibodies and washed them in PBS. Then dried and mounted them with Vectashield Hard Set Mounting Medium with DAPI for VGF and BDNE, or with Gel/Mount aqueous mounting medium with anti-fading agents (Biomed) for the double-immunohistochemical studies (VGF and NeuN, VGF and synaptophysin, and VGF and PSA-NCAM)³⁹. See the **Supplementary Methods** for details on primary and secondary antibodies used.

Immunohistochemistry semiquantitative comparison. We took immunohistochemical images of VGF, exported them as 16-bit grayscale TIFF images, and then quantified them with US NIH image software. We used a drawing tool to outline the dentate gyrus as our region of interest. We used medium- to low-intensity images that allowed for the quantification to be conducted in a linear densitometric range. By using this approach, we were able to increase our dynamic range along a linear scale to more accurately quantify the regulation of VGF protein.

Equipment and settings. We acquired immunohistochemistry images on a Zeiss Axioskop 2 microscope with AxioVision 3.1 software; images for each protein were taken under the same exposure conditions.

Cannulation surgery in mouse and rat. We performed all surgeries under aseptic conditions and under anesthesia (see the **Supplementary Methods** for details).

Behavioral testing. For free wheel running, we housed running and sedentary mice in standard plastic cages (12 × 30 × 13 cm) with *ad libitum* access to food and water.

For immobilization stress, we subjected Sprague-Dawley rats (300–350 g) to 45 min of immobilization stress as described previously⁴² and then placed them back into their home cages. Two hours later, we killed the rats and harvested their brains for VGF and BDNF *in situ* hybridization analysis.

The forced-swim test and the tail-suspension test are well-established tests of depression and antidepressant response, and we conducted them according to standard laboratory procedures. We assessed locomotor activity to determine the specificity of the antidepressant effects of VGF (0, 0.2, 1 and 5 µg) infusions. All vehicle or VGF infusions were given 4 h before behavioral testing (see **Supplementary Methods** for details of behavioral analysis).

The novelty-induced hypophagia model consists of 3 d of habituation, a home-cage test, and then a novel-cage test in which latency to drink from the sweetened milk bottles is scored (see the **Supplementary Methods** for details).

We measured anxiety-related behavior was measured with the elevated-plus-maze and open-field tests (see the **Supplementary Methods** for details).

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

We would like to thank S. Salton (Mount Sinai School of Medicine) for providing us with male C57BL/6J mice; J.W. Koo for his assistance with the restraint studies; J.W. Koo and J. Quinn for their assistance with the statistical analysis; and K. Patterson and C. Montgomery for assistance in breeding and genotyping VGF-mutant mice. We would like to acknowledge the support of the National Research Service Award fellowship, US National Institute of Mental Health grants MH25642 and MH45481, US NIH grants DK57702 and NS45305, the US National Alliance for Research on Schizophrenia and Depression grants DK-071308 and U24 NS05186, and the Connecticut Mental Health Center.

AUTHOR CONTRIBUTIONS

J.G.H. assisted with all aspects of the research, including optimization of the microarray and data analysis, conducted all other molecular and behavioral experiments, and prepared the original draft of manuscript. S.S.N. was responsible for the development, optimization and experimental use of custom array. A.H.B. assisted in the optimization, use and analysis of microarrays. C.H.D. assisted in development of the running procedure and behavioral analysis. D.S.R. assisted in analysis of the microarray data, PC12 culture work and discussion of results. S.R.S. assisted in conceptual aspects of the studies, the development of VGF-mutant mice and interpretation of the data. R.S.D. was involved in the development of the overall study design, data analysis, interpretation of results and the preparation of manuscript and figures. All authors discussed results and contributed intellectually to the manuscript.

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