

Nerve growth factor (NGF) has novel antidepressant-like properties in rats

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ABSTRACT

Nerve growth factor, a neurotrophin, may have other functions, including a role in depressive disorders. The present study sought to determine whether NGF would (1) have antidepressant-like effects and (2) behave similarly to or differently from other well-recognized antidepressants. Over a broad dose-range, NGF reduced the exaggerated swim test immobility exhibited by the Flinders Sensitive Line (FSL) rats, but at a standard dose of 40 ng/ml, it was not as effective as desipramine (DMI, 5 mg/kg). The low social interaction behavior and locomotor activity of the FSL rats were less affected by NGF than was the immobility. Acute treatment with NGF did not induce c-fos expression in brain regions known to be activated by other acute antidepressants. The fact that chronic treatment with DMI blunted the corticosterone response to fluoxetine was replicated in this study. However, chronic treatment with NGF did not alter this response. Similarly, chronic treatment with fluoxetine blunted 5-HT_{1A} and 5-HT_{2A} receptor-mediated responses, whereas chronic treatment with NGF was without effect. Thus, NGF has antidepressant-like effects but does not appear to have biochemical actions typical of other antidepressants.

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Neurotrophins, including nerve growth factor (NGF) and brain-derived nerve growth factor (BDNF), are recognized to play roles other than their classical trophic effects (Altar, 1999; Duman, 2002). One such function that has become increasingly investigated is a role in depressive-like behavior. Work has been particularly active with regard to BDNF, with demonstrations that it is elevated in rats undergoing antidepressant treatments (Chen et al., 2001), that it may have antidepressant-like effects after central injections (Shirayama et al., 2002; Siuciak et al., 1997), and that depressed individuals have a deficiency of BDNF (Karege et al., 2002; Shimizu et al., 2003). Much less attention has been devoted to the potential involvement of NGF in depressive-like behavior and antidepressant action, although there are some supporting data. A key finding is that although both NGF and BDNF are reduced in certain brain regions of the Flinders Sensitive Line (FSL) rat, a genetic animal model of depression (Angelucci et al., 2000, 2004; Overstreet et al., 2005), the application of electroconvulsive shock, a robust antidepressant treatment in rats, elevated NGF only (Angelucci et al., 2003). Anecdotal experience with dogs suffering from separation anxiety

and treated with a formulation of low concentration NGF indicated that they exhibited improved behavior as a consequence of therapy (unpublished observations, 2002). Therefore, there was an interest in systematically evaluating the antidepressant potential of NGF in preclinical animal models.

The FSL rat is innately more immobile in the forced swim test than its control counterpart, the Flinders Resistant Line (FRL) rat, and exhibits a decrease in immobility following chronic, but not acute, treatment with desipramine (DMI) or sertraline (Pucilowski and Overstreet, 1993). The FSL rat exhibits other features that are similar to those found in human depressives, such as increased REM sleep (Benca et al., 1996; Shiromani et al., 1988), has serotonergic abnormalities that are corrected following chronic antidepressant treatments (Zangen et al., 1997), and responds to other antidepressants that appear to be clinically useful (see Overstreet, 2002, 2005).

It is important to know not only whether NGF will have antidepressant-like effects but also whether the mechanisms underlying these effects are similar to or different from those of the classical antidepressants. Consequently, three paradigms were examined in FSL or normal outbred rats: 1) regional brain c-fos expression after injection of NGF and/or exposure to the swim test (Duncan et al., 1996); 2) induction of corticosterone elevation by acute fluoxetine in rats chronically treated with NGF or DMI (Duncan et al., 1998); 3) induction of hypothermia or head shakes by serotonin agonists in rats chronically treated with NGF or fluoxetine (Albert et al., 1996; Gray and Roth, 2001; Stahl, 1994; Toth, 1996).

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1. Methods

1.1. Antidepressant experiments

In preliminary studies, NGF was compared with vehicle or other ingredients in a medicine; NGF significantly reduced immobility while none of the other ingredients had a significant effect (unpublished findings, 2001). These encouraging findings led to the dose-response study described here.

1.1.1. Animals

The FSL and FRL rats were selected from breeding colonies maintained at the University of North Carolina Bowles Center for Alcohol Studies. They were housed in groups of three in temperature- and humidity-controlled rooms under a 12:12 light:dark cycle (lights on 0700–1900). Rats were randomly divided into nine FSL groups and one FRL reference group and then given the treatments described below.

1.1.2. Research design

The basic design included the following treatment groups: FRL rats given vehicle; FSL rats given vehicle; FSL rats given DMI as a positive control; and FSL rats given various doses of NGF. The concentration of NGF was altered in the solution so that every rat received an injection of 0.2 ml/kg, regardless of weight. The concentrations of the NGF solutions were 2.96, 14.8, 64, 320 pg/ml and 1.6, 8, 40 (standard dose) and 80 ng/ml. The weights of the rats did not differ by more than 20 g, so the doses did not differ.

1.1.3. Chronic treatment of NGF

FSL rats ($n=8$ or more) were divided into 9 treatment groups (vehicle and 8 different doses of NGF). Rats were injected s.c. with 0.2 ml of the allocated dose for 14 consecutive days. Approximately 20 h after the last treatment the rats were placed in the social interaction arena for a 5-min session. Approximately 90 min later, they were placed individually in the swim tank for a 5-min recording of swimming behavior. A saline-treated Flinders Resistant Line (FRL) group ($n=8$), which swims significantly more than the FSL rats, was tested in the two behavioral tasks as a reference group. A positive control FSL group was also used; DMI (5 mg/kg) was injected for 14 consecutive days and the behavioral tests were conducted between 21 and 23 h after the last injection.

In a separate study, 24 FSL rats were used to attempt a replication of the findings with NGF. The three treatment groups were vehicle, 40 ng NGF and 5 mg/kg desipramine. After 14 days of daily treatments, the rats were tested in the social interaction and forced swim tests.

1.1.4. Behavioral tests

Approximately 20 h after the last treatment, rats with similar body weights were placed in a square test arena (60×60 cm, with 16 15×15 cm squares marked on the floor) for the testing of social interaction. The amount of time spent in social interaction (grooming, licking, sniffing, and crawling over or under) was recorded during a 5-min session by an experienced observer who was blind to the treatment condition. This measure provides one index of anxiety-like behavior, with more “anxious” rats spending less time in social interaction (File and Seth, 2003; Overstreet et al., 2003). In addition, a motor activity measure was collected. The total number of lines crossed during the session provided a measure of general activity.

The swim tank was 18 cm in diameter and 40 cm tall. The tank was filled with enough 25 °C water so the rat could not touch the bottom with its feet. The rat was placed in the swim tank for a single 5-min session 21–23 h after the last treatment and the seconds of immobility was scored by an observer blind to the treatment condition and rat strain being tested (Overstreet, 1993; Zangen et al. 1997).

1.1.5. Statistical analyses

The data for the three measures in the chronic treatment study were summarized into mean ± s.e.m. for each of the 10 treatment groups. For the swim test the immobility scores were converted to swimming scores to make it consistent with the other behavioral measures, where the score for the FSL rat is lower than the score for the FRL rat. Graphical representations of the findings were compiled using Prism software. Initially, the data for each measure were subjected to one-way ANOVAs. If the ANOVA revealed significant group differences, follow-up Tukey's tests were carried out to elucidate the pattern of group differences. The GBstat software package was used for the statistical analyses.

1.2. *c-fos* expression

The research design evaluated the acute and chronic effects of NGF (standard dose of 40 ng/ml) on the regional brain expression of *c-fos*, an early/immediate gene product that is associated with increased activity in the brain (e.g., De Medeiros, 2005; Duncan et al., 1996; Morinobu et al., 1995, 1997). Rats were either injected acutely with the standard dose of NGF ($n=4$) or vehicle ($n=4$) or chronically for 14 consecutive days with the same doses of NGF ($n=8$) or vehicle ($n=8$). The rats were briefly handled on two occasions prior to the start of the injections to minimize the effects of injection stress. The rats were sacrificed 2 h after the acute injections, intracardially perfused with 4% paraformaldehyde and the brain removed and stored until processed for *c-fos* expression (Duncan et al., 1996). In the chronic treatment groups, the rats were exposed to the swim tank between 22 and 24 h after the last injection and then sacrificed 2 h later and the same procedures as for the acutely treated rats were followed.

c-fos expression was assessed by immunocytochemistry, following the methods developed by Duncan et al. (1996). For all conditions, rats were anesthetized with 70 mg/kg of pentobarbital sodium 2 h after initiation of the different procedures and perfused transcardially at a rate of 35–40 ml/min with ice-cold 100 mM sodium phosphate-buffered saline for 4 min (PBS, pH = 7.4), followed by 4% paraformaldehyde in phosphate buffer (PB) for 7 min. Brains were post-fixed overnight in 4% paraformaldehyde in PB. Numerous studies have shown that a 2 h delay following presentation of a variety of stimuli is optimal for immunocytochemical analysis of Fos in the brain (Malberg et al., 2000; for review see [Slattery et al., 2005]). Vibratome sections of brain (60 μm) were cut and placed in PBS contained in 24-well tissue culture dishes. Sections were treated with 10% normal rabbit serum and 0.2% Triton X-100 in PBS for 30 min and then were then incubated for 48–72 h at 4 °C with a polyclonal Fos antiserum raised in rabbits (1:20,000 dilution; Biogenesis, Brentwood, NH). The antibody was raised against a synthetic peptide corresponding to conserved regions of mouse and human Fos. The antibody generated exclusively nuclear specific staining. Since it is uncertain whether the antibody recognizes Fos-related antigens as well as Fos, results are described as Fos-like immunoreactivity (Fos-LI). However, in previous work, staining with the antisera was maximal at 2 h, was reduced substantially by 4 h, and was not different from controls by 6 h (Malberg et al., 2000). Thus, since the induction of Fos-related antigens is delayed relative to Fos and persists well beyond 6 h after a stimulus (Slattery et al., 2005), the time course of staining with the antibody is consistent with a recognition of only Fos. After incubation with the Fos antiserum, sections were processed through 3 rinses of PBS and incubated for 1 h with biotinylated rabbit-antigoat antibody (Vector Laboratories, Burlingame, CA). After 3 rinses with PBS the sections were incubated with avidinbiotin complex (Vector Laboratories) for 1 h. With an additional 3 rinses with PBS, sections were placed in a solution containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB), 0.005% cobalt chloride, 0.007% nickel ammonium sulfate, and 0.003% hydrogen peroxide. For each animal, anatomically intact/high

quality regions from both left and right sides of the brain where possible were isolated and all Fos-LI-positive cells within a defined area (Table 2) were counted. Mean cell counts for each animal/region were used to calculate differences across groups.

Cells exhibiting Fos-LI in selected brain regions were counted in a circular field defined by the area of section viewed with a 10× or 20× objective, depending on the size of the region of interest. In other brain regions, the number of cells within defined histological regions was counted. This approach of cell counting was designed to accurately reflect the number of cells within specific brain regions.

1.2.1. Statistical analyses

The data for c-fos expression were analyzed separately for each brain region. Initially, the acute and chronic studies were treated as independent and *t* tests were used to compare the vehicle- and NGF-treated groups. However, because the acute groups were not exposed to the swim test and the chronic groups were, the effects of exposure to the swim tank could also be evaluated. Therefore, a global 2-way ANOVA was carried out, with swim test exposure and NGF treatment as the two independent variables.

1.3. Blunted corticosterone response as antidepressant effect

Fluoxetine acutely stimulates blood corticosterone levels (Duncan et al., 1998). However, the effects of fluoxetine are reduced in rats chronically treated with antidepressants, including DMI (Duncan et al., 1998). Consequently, the response to fluoxetine was determined in rats chronically treated with NGF and compared with rats chronically treated with DMI.

1.3.1. Animals

Outbred Sprague–Dawley rats were used for this study because most of the previous studies used this strain (e.g., Duncan et al., 1998). Thirty rats were purchased from Charles-River (Raleigh, NC) at about 70 days of age. They were divided into three equal groups of 10 (vehicle treatment, NGF treatment, and DMI treatment).

1.3.2. Procedure

Rats were treated for 21 consecutive days with either vehicle (4 ml/kg), 4 ng NGF (0.2 ml/rat), or 15 mg/kg DMI (4 ml/kg). Approximately 22 h after the 18th injection, the rats were placed in cylinders filled with 25 °C water and immobility was recorded by an observer blind to treatment during a single 5-min session. After the test, the rats were dried thoroughly and returned to their home cages. The daily scheduled treatment was given about 90 min later. At 22–24 h after the last treatment (21st), the rats were injected i.p. with 5 mg/kg fluoxetine. Forty min later the rats were sacrificed by decapitation, blood collected and plasma samples stored at –80 °C until assays of corticosterone could be carried out, using a radioimmunoassay kit (Sigma).

1.3.3. Statistical analysis

Data were analyzed initially by one-way ANOVAs. When these revealed significant differences, Tukey's protected *t* tests were carried out to determine the pattern of group differences.

1.4. Blunted 5-HT_{1A} and 5-HT_{2A} responses as antidepressant effect

A functional down-regulation of 5-HT_{1A} and 5-HT_{2A} receptor responses has been commonly observed in rats treated chronically with antidepressants, particularly selective serotonin reuptake inhibitors (Stahl, 1994; Toth, 1996). Because these responses may or may not be accompanied by parallel decreases in the respective receptors (Albert et al., 1996; Gray and Roth, 2001), we decided to use functional assays in this study. Specifically, hypothermia was used to assess 5-HT_{1A} receptor function (Overstreet et al., 1994) and head

shakes were used to assess 5-HT_{2A} receptor function. It was predicted that these responses would be blunted after chronic treatment with fluoxetine (e.g. Albert et al., 1996; Gray and Roth, 2001) and that they would also be blunted after NGF treatment if NGF were acting like fluoxetine.

1.4.1. Animals

Outbred Sprague–Dawley rats were used because these rats were the primary group used in previous studies of 5-HT_{1A} and 5-HT_{2A} receptor function. Two groups of 30 rats were obtained, one batch to study 5-HT_{1A} receptor function and one batch to study 5-HT_{2A} receptor function.

1.4.2. Procedure

Rats were chronically treated for 21 consecutive days with either vehicle, 5 mg/kg fluoxetine (1 ml/kg) or 4 ng NGF (0.2 ml/rat). At about 22 h after the 18th injection the rats were challenged with 0.25 mg/kg 8-OH-DPAT, a 5-HT_{1A} receptor agonist, or 5 mg/kg DOI, a 5-HT_{2A/C} receptor agonist. Temperature was recorded 45 min after the 8-OH-DPAT injection and related to previously recorded baselines. Headshakes were scored in the DOI-treated rats continuously between 15 and 45 min after the injections. The rat's normal scheduled treatment was given about 1 h after the challenge treatments. Chronic treatment continued for two more days and then the swim test was conducted about 22 h after the last (21st) injection.

1.4.3. Statistical analysis

Data were analyzed initially by one-way ANOVAs. When these revealed significant differences, Tukey's protected *t* tests were carried out to determine the pattern of group differences.

2. Results

2.1. Chronic NGF treatment

The results are summarized in Figs. 1 and 2 (swimming), 3 and 4 (social interaction) and 5 and 6 (line crossings). For each variable, the first figure of the pair compares the standard dose of NGF with FRL rats and FSL rats treated with vehicle or DMI, a positive control. The second figure of the pair illustrates the findings from all of the NGF treatments. Thus, Fig. 1 shows that while NGF increases swimming significantly in the FSL rats, the effect is not as robust as that for DMI because FSL rats treated with DMI are not significantly different from the FRL rats. In other words, DMI at a dose of 5 mg/kg/day for 14 days

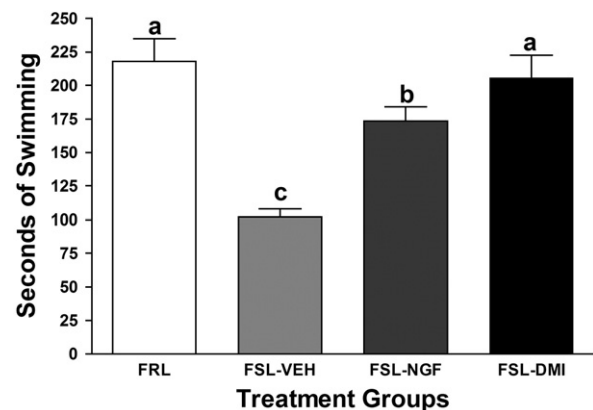


Fig. 1. The effects of chronic treatment with desipramine (DMI, 5 mg/kg/day) or NGF (40 ng/kg/day) on swim test behavior in Flinders Sensitive Line (FSL) rats. Drugs were injected daily for 14 days. The 5-min swim test was conducted approximately 23 h after the last treatment. The control FRL rats were also given vehicle. The data represent the mean ± s.e.m. seconds swimming for 8–10 rats.

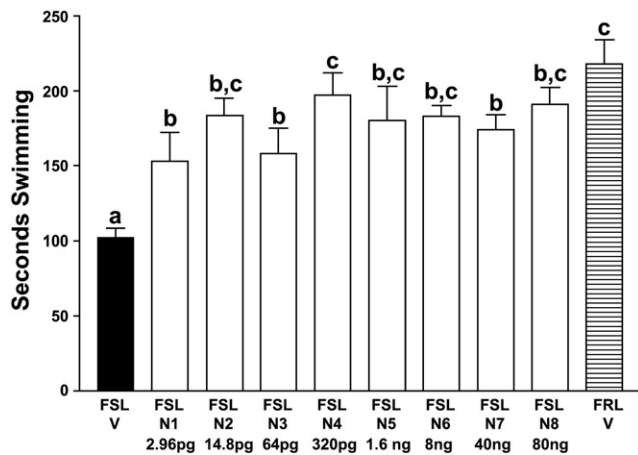


Fig. 2. The effects of different doses of nerve growth factor on swimming in the forced swim test in FSL rats. Drugs were injected daily for 14 days. The 5-min swim test was conducted approximately 23 h after the last treatment. The control FRL rats were given vehicle also. The data represent the mean \pm s.e.m. seconds of swimming for 8–10 rats. Groups with the same letter are not significantly different from each other.

completely normalized the behavior of the FSL rats, but NGF did not. Fig. 2 shows that, as reported in Fig. 1, FSL rats treated with vehicle and the standard dose of NGF (40 ng/ml) were significantly different from the FRL rats (although they are also different from each other). Overall, there was a significant treatment effect ($F[9,83]=6.66$, $p<0.001$). Like 40 ng/ml, the doses of 2.96 and 14.8 pg/ml failed to normalize fully the behavior of the FSL rats. However, the other five treatments induced swimming scores in the FSL rats that were not significantly different from the scores of the FRL rats.

The results in Fig. 1 are consistent with the findings above that the standard dose of NGF has only a partial counteracting effect on the exaggerated swim test immobility. However, for social interaction, NGF did not have a significant effect at all on the abnormally low social interaction in the FSL rats. In contrast, chronic treatment with DMI increased social interaction behavior in the FSL rats to the level of the FRL rats (Fig. 3). Thus, DMI normalized both behaviors in the FSL rats, whereas the standard dose of NGF only partially normalized the abnormal swimming behavior. As shown in Fig. 4, all of the NGF treatments slightly increased social interaction behavior above that seen in the vehicle-treated FSL rats. However, no single treatment produced social interaction behavior similar to that observed in the FRL rats (Fig. 4). Indeed, the one-way ANOVA indicated that there were no significant differences ($F[9,83]=1.25$, NS).

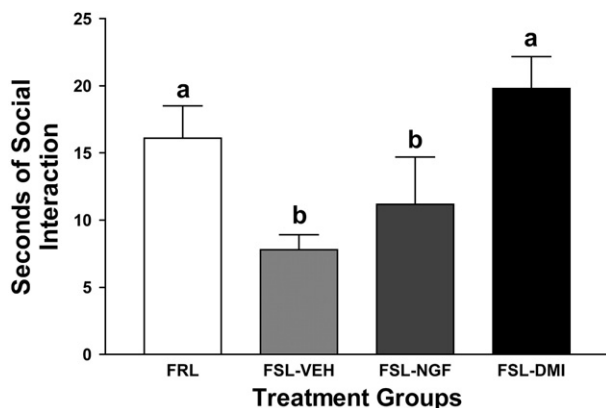


Fig. 3. Social interaction behavior in FSL rats after chronic treatment with desipramine (DMI, 5 mg/kg/day) or NGF (40 ng/kg/day). Drugs were injected daily for 14 days. The 5-min social interaction test was conducted approximately 22 h after the last treatment. The control FRL rats were also given vehicle. The data represent the mean \pm s.e.m. seconds of social interaction for 8–10 rats. Groups with different letters are significantly different according to Tukey's tests.

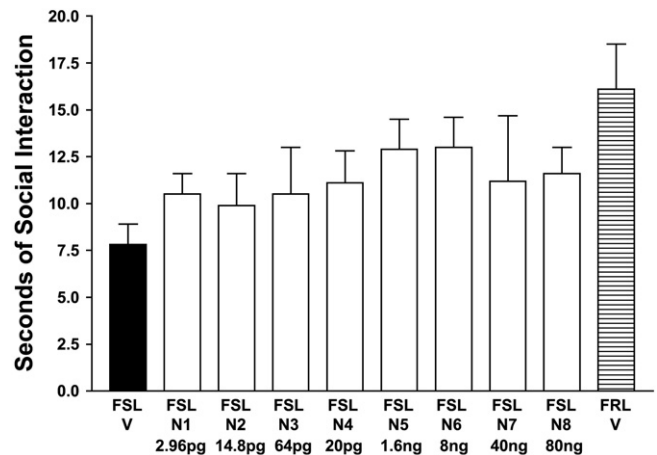


Fig. 4. The effects of different doses of NGF on social interaction behavior in FSL rats. Drugs were injected daily for 14 days. The 5-min social interaction test was conducted approximately 22 h after the last treatment. The control FRL rats were also given vehicle. The data represent the mean \pm s.e.m. seconds of social interaction for 8–10 rats.

The results for locomotor activity, illustrated in Figs. 5 and 6, present a different pattern than for swimming and social interaction. Here the standard dose of NGF increased the activity of the FSL rats, whereas DMI did not. Still, the activity of the FRL rats was even higher (Fig. 5). As shown in Fig. 6, there were significant differences among the various treatments ($F[9,83]=6.70$, $p<0.001$). The two lowest doses of NGF also increased activity, but the other ones did not.

The fact that the NGF did increase activity under some conditions could suggest that the effects on swimming are merely secondary to the effects on activity. To investigate this further, analyses of covariance were carried out on immobility and activity. When activity scores were controlled in the initial study, there was no significant effect on swimming ($F[3,32]=0.95$, NS). However, when activity scores were controlled in the second study, a highly significant effect of treatment on immobility remained ($F[9,129]=17.37$, $p<0.001$). Thus, swim test immobility is not simply dependent upon locomotor activity.

The results of the replication study, reported in Table 1, supported the above conclusion. There were no significant differences for social interaction ($F[2,20]=0.21$, NS) or line crosses ($F[2,20]=0.50$, NS), but the differences in immobility were highly significant ($F[2,20]=47.7$, $p<0.0001$). Furthermore, an analysis of covariance using

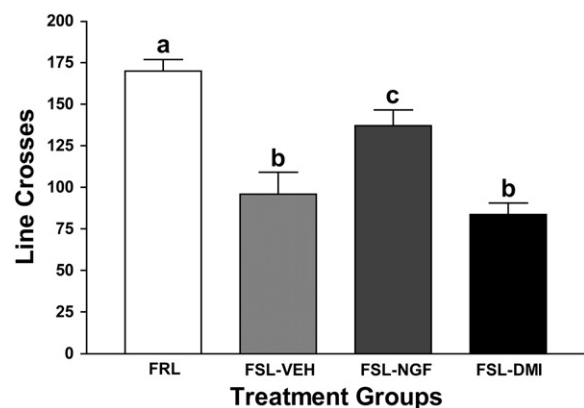


Fig. 5. The effects of chronic treatment with desipramine (DMI, 5 mg/kg/day) or NGF (40 ng/kg/day) on line crossings in the social interaction test in FSL rats. Drugs were injected daily for 14 days. The 5-min social interaction test was conducted approximately 22 h after the last treatment. The control FRL rats were also given vehicle. The data represent the mean \pm s.e.m. lines crossed for 8–10 rats. Groups with different letters are significantly different according to Tukey's tests.

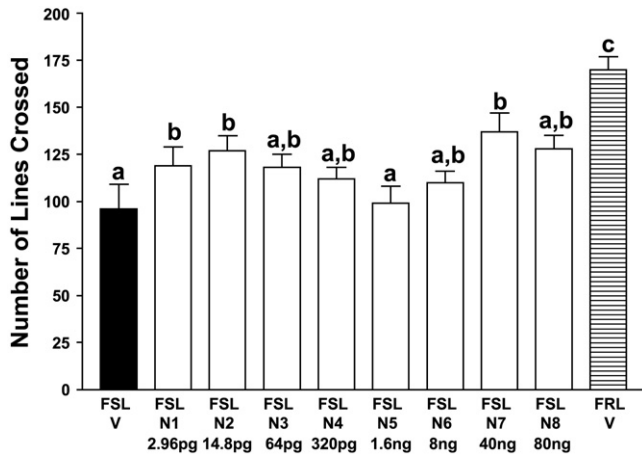


Fig. 6. The effects of different doses of NGF on line crossings in FSL rats. Drugs were injected daily for 14 days. The 5-min social interaction test was conducted approximately 22 h after the last treatment. The control FRL rats were also given vehicle. The data represent the mean \pm s.e.m. lines crossed for 8–10 rats. Groups with different letters are significantly different according to Tukey's tests.

locomotor activity as the covariate still revealed a highly significant effect on immobility ($F[2,20] = 49.04, p < 0.0001$).

2.2. *c-fos* expression

The results are summarized in Table 2. A wide range of brain regions was sampled and two-way ANOVAs were used to analyze the data in each region. Despite the number of brain regions sampled, there were no significant effects of either acute or chronic NGF treatment (all *F*, NS). Even in the four brain regions (Table 2) where there was a significant effect of swim test exposure (PVN, $F[1,16] = 70.47$; central amygdala, $F[1,16] = 11.14$; medial amygdala, $F[1,16] = 11.72$; and shell of the nucleus accumbens, $F[1,16] = 10.63$), NGF treatment did not modify the effects of the swim test. Thus, NGF does not appear to induce brain *c-fos* expression, nor does it modify expression induced by swim test exposure.

2.3. Blunted corticosterone response after chronic antidepressant treatment

The results are summarized in Fig. 7. In the top panel of the figure are the scores for swim test immobility. The vehicle-treated rats are significantly more immobile than either the NGF- or DMI-treated rats ($F[3,28] = 15.18, p < 0.001$). Both NGF and DMI had antidepressant-like effects. In contrast, as shown in the bottom panel of Fig. 7, only chronic DMI treatment induced a blunting of the corticosterone response to fluoxetine. NGF was without effect. A one-way ANOVA confirmed the group differences ($F[3,26] = 3.84, p < 0.05$) and Tukey's protected *t* tests established that the DMI-treated group was significantly different from the other three groups.

Table 1
Replication of effects of NGF and DMI on swim test immobility.

Social interaction			Line crosses			Immobility		
Vehicle	NGF	DMI	Vehicle	NGF	DMI	Vehicle	NGF	DMI
14 \pm 3	13 \pm 4	11 \pm 3	77 \pm 20	79 \pm 7	62 \pm 8	219 \pm 6	166 \pm 5 ^a	130 \pm 8 ^a

There were 8 rats in each group. Tests were conducted 21–23 h after 14 consecutive daily injections of 40 ng NGF, 5 mg/kg DMI, or vehicle.

^a Significantly different from vehicle treatment, $p < 0.05$, Tukey's protected *t* test.

2.4. Blunted 5-HT_{1A} and 5-HT_{2A} responses after chronic treatment

The results are summarized in Table 3. In the first column the data for swim test immobility are shown. There were significant group differences ($F[2,26] = 40.24, p < 0.0001$) and both fluoxetine and NGF significantly reduced immobility. However, both the number of head shakes after DOI, a 5-HT_{2A/C} agonist, and the degree of hypothermia after 8-OH-DPAT, a 5-HT_{1A} agonist, were reduced only in the fluoxetine-treated rats. Tukey's tests confirmed that the fluoxetine-treated rats were significantly different from the other two groups. Thus, chronic treatment with NGF produces a similar antidepressant-like response as does fluoxetine, but it does not induce blunting of serotonergic responses.

3. Discussion

The present findings may be the first to report that injected NGF has an antidepressant-like effect; a preliminary report of these findings has been presented (Overstreet et al., 2004a). The reduction in immobility after chronic NGF treatment was seen under a variety of conditions. Several different doses of NGF reduced the exaggerated immobility (increased swimming) of the FSL rat and they tended not to counteract the low social interaction behavior and had mixed effects on the reduced locomotor activity. Furthermore, chronic treatment with NGF reduced immobility in outbred Sprague–Dawley rats to an extent similar to fluoxetine but not as strong as DMI. These findings are consistent with the suggestions of others that NGF may play a role in depression (Angelucci et al., 2000, 2003). It is quite remarkable that these antidepressant effects can be observed with such low doses. The standard concentration of NGF, which was used for most of these studies, was 40 ng/ml. With the rats in these studies weighing an average of 400 g, a dose of 100 ng/kg was used. Most other antidepressant compounds require higher doses; for example, DMI and fluoxetine are typically administered in a dose of 5 mg/kg (Overstreet et al., 2005) and others may require 10 mg/kg or more (Overstreet and Griebel, 2004; Overstreet et al., 2004b).

The fact that NGF treatment also increases locomotor activity under some conditions raises the possibility that the reduction in immobility is a simple consequence of the stimulant effects of NGF. However, that cannot be the case, as there were several instances where immobility was reduced without an effect on locomotor activity. Data from other studies reinforce the view that antidepressant effects on locomotor activity are independent of those on immobility. In a recent study, for example, both citalopram and DMI reduced swim test immobility in the FSL rats, but only citalopram increased locomotor activity (Overstreet et al., 2004b). Earlier on, it was reported that the psychomotor stimulants like amphetamine and scopolamine do not decrease immobility (Overstreet et al., 1995). A key point about the present data is that NGF treatment consistently reduced immobility under a variety of conditions but the effects on locomotor activity are only seen occasionally.

To integrate these results we can describe the profile for DMI and then determine which, if any, of the NGF treatments resemble this profile. As indicated earlier, DMI increases swimming to the level of the FRL rats (Fig. 1) and also increases social interaction (Fig. 3), but does not affect locomotor activity (Fig. 5). 2.96 pg and 14.8 pg of NGF normalize swimming (Fig. 2); but do not increase social interaction (Fig. 4) or locomotor activity (Fig. 6). Drugs that increase activity are not looked upon favorably as potential antidepressants because of possible nonspecific stimulant effects. Therefore, the robust effects on swimming of 8 and 40 ng of NGF are problematic. However, this increase is still below the activity exhibited by the FRL rats (Fig. 6), so it may be a partial normalization. Under this view, 2.96 pg and 8 and 40 ng of NGF would be the best because they tend to normalize each of the abnormal behavioral responses of the FSL rats.

Table 2
Regional brain c-Fos expression after acute or chronic treatment with NGF or vehicle.

Brain region	Bregma level ^a	Grid used ^b	Total magnification ^c	Number of Fos positive cells			
				Acute treatment		Chronic treatment + swim test	
				Vehicle	NGF	Veh	NGF
MPCx	2.7	10×10	100×	137 ± 24	171 ± 33	206 ± 26	190 ± 15
Cingulate Cx	1.2	10×10	200×	48 ± 7	29 ± 4	49 ± 6	45 ± 7
PVN	−1.88	10×10	200×	47 ± 8	43 ± 13	132 ± 12 ^d	155 ± 7 ^d
Central amygdala	−2.8	10×10	200×	9 ± 3	5 ± 1	18 ± 2 ^d	17 ± 3 ^d
Lateral amygdala	−2.8	10×10	100×	14 ± 4	15 ± 7	19 ± 4	20 ± 2
Basolateral amygdala	−2.8	10×10	200×	21 ± 3	22 ± 3	25 ± 2	24 ± 3
Medial amygdala	−2.8	5×10	100×	63 ± 12	41 ± 11	89 ± 9 ^d	98 ± 10 ^d
N.Ac. shell	1.2	5×10	200×	18 ± 6	12 ± 7	33 ± 4 ^d	26 ± 3 ^d
N.Ac. core	1.2	10×10	200×	9 ± 5	2 ± 1	14 ± 3	10 ± 2
Frontal Cx	1.2	10×10	200×	64 ± 9	53 ± 18	68 ± 7	63 ± 12
Dentate	−3.3	10×10	200×	17 ± 3	15 ± 3	15 ± 3	17 ± 2
Clastrum	2.7	10×10	200×	53 ± 14	48 ± 22	54 ± 10	59 ± 13

Abbreviations: MPC – medial prefrontal cortex, prelimbic subregion; Cx – cortex; PVN – paraventricular nucleus of the hypothalamus; N. Ac. – nucleus accumbens.

^a Based on the atlas of Paxinos and Watson 4th Edition.

^b A 10×10 or 5×10 eyepiece grid was used to isolate the brain region or a portion thereof, then all cells within the grid pattern were counted if the region being counted exceeded the grid chosen. Or, if the brain region was smaller than the grid chosen based on the atlas of Paxinos and Watson, then the entire region was counted.

^c Combined objective and eyepiece magnification.

^d Significantly different from acute vehicle treatment, $p < 0.05$, Tukey's test.

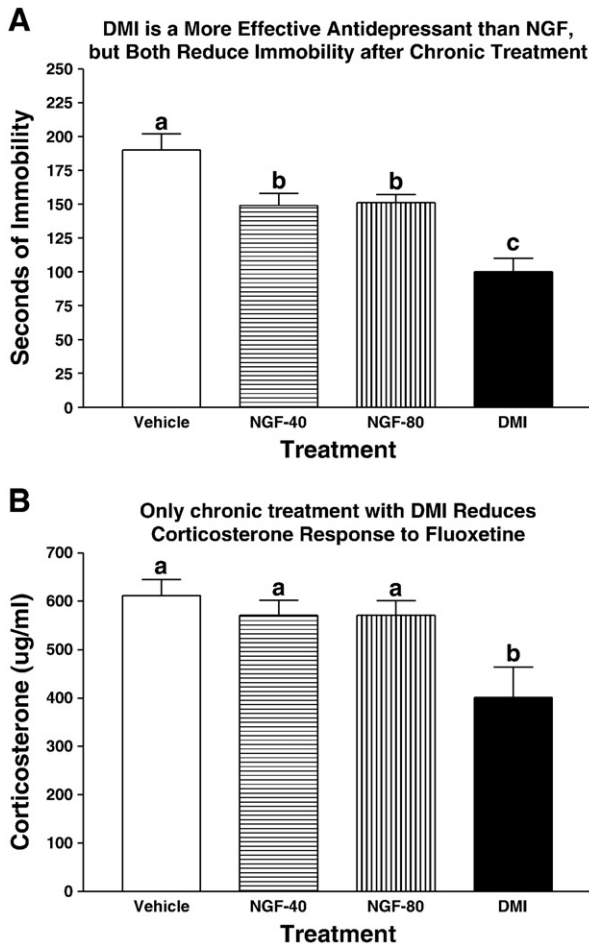


Fig. 7. The effects of chronic treatment with desipramine (15 mg/kg/day) of NGF (40 or 80 ng/kg/day) on corticosterone response to fluoxetine. Drugs were given ip chronically for 18 days. Fluoxetine (5 mg/kg) was administered 22 h after the 18th injection and blood samples were taken 40 min later for the analysis of corticosterone. The data represent the mean ± s.e.m. µg/ml of corticosterone for 8–10 rats and are presented in the bottom panel. Treatment continued for a further three days and about 21–23 h after the last treatment the rats were tested in the swim test. The data represent the mean + s.e.m. seconds of immobility and are presented in the top panel.

In an attempt to elucidate the basis of the antidepressant effects of NGF, we examined three paradigms that have been commonly used to investigate the mechanisms of action of antidepressants. The first was regional brain c-fos expression. This measure was recorded both after acute treatment with NGF and after chronic treatment in which the animals were sacrificed 2 h after exposure to the swim tank. Neither of these procedures provided any evidence that NGF was acting like typical antidepressants. Both Beck (1995) and Dahmen et al. (1997) reported that a variety of antidepressants increased c-fos expression in the central amygdala following acute administration. Yet there was no such change after acute treatment with NGF (Table 2).

The present study included groups that were chronically treated with NGF at a dosage schedule that reduced swim test immobility. Despite the significant behavioral difference between the NGF- and vehicle-treated rats, they exhibited similar c-fos expression (Table 2). In contrast, other antidepressants alter the c-fos response to swim stress following chronic treatment (Duncan et al., 1996). Thus, NGF appears to be an antidepressant without the biochemical properties associated with other compounds. Although the rats were handled prior to the acute injections, there is still the possibility that the effects of injection stress obscured any effects of NGF itself on c-fos expression. All we can conclude is that the effects of NGF were not different from the effects of saline vehicle. However, other studies have concluded that the effects of other antidepressants on c-fos expression do differ from saline vehicle (Beck, 1995; Dahmen et al., 1997; Duncan et al., 1996).

Another paradigm that has been utilized in an attempt to clarify the mechanism of action of antidepressants is fluoxetine-induced elevation of corticosterone. Duncan et al. (1998) demonstrated that the corticosterone response to acute fluoxetine treatment was

Table 3
Effects of chronic treatment with fluoxetine or NGF on swim test immobility and responses to 5-HT_{1A} and 5-HT_{2A} receptor agonists.

Treatment group	Immobility (seconds)	Hypothermia (°C)	Head shakes (number)
Vehicle	221 ± 6 ^a	−1.75 ± 0.15 ^a	4.8 ± 1.1 ^a
NGF	162 ± 6 ^b	−1.71 ± 0.12 ^a	4.8 ± 1.3 ^a
Fluoxetine	146 ± 7 ^b	−0.74 ± 0.06 ^b	1.9 ± 0.6 ^b
F [2,26]=	40.24***	22.77***	3.40*

* $p < 0.05$; *** $p < 0.001$. Groups with different letters are significantly different.

blunted following chronic treatment with a variety of antidepressants. The present study confirmed that chronic treatment with DMI would blunt the corticosterone response to fluoxetine (Fig. 7). However, chronic treatment with NGF had no such effect. At the same time, this study confirmed the antidepressant-like effects of NGF (Fig. 7). Thus, other antidepressants are behaving differently from NGF.

The last paradigm to be considered is the functional down-regulation of 5-HT_{1A} and 5-HT_{2A} receptors following chronic treatment with antidepressants (Albert et al., 1996; Gray and Roth, 2001; Overstreet et al., 2005; Stahl, 1994; Toth, 1996). The present report confirmed previous observations regarding fluoxetine: chronic treatment with this SSRI blunted the hypothermic response to 8-OH-DPAT, a 5-HT_{1A} receptor agonist, as well as a reduced behavioral response to DOI, a 5-HT_{2A/C} receptor agonist (Table 3). However, the responses to these two agonists were unchanged in rats chronically treated with NGF (Table 3), despite both compounds reducing swim test immobility. Thus, although NGF clearly has antidepressant-like properties, it does not induce a biochemical profile similar to fluoxetine.

The data presented in this paper allow us to suggest that NGF is a novel antidepressant because it does not alter c-fos expression in a way similar to other antidepressants or induce a blunting of the corticosterone response to fluoxetine or a down-regulation of 5-HT_{1A} or 5-HT_{2A} receptors. What about other mechanisms that might be involved?

An important consideration is whether NGF is having its antidepressant properties via actions in the brain or in the periphery. The conventional wisdom is that NGF does not readily penetrate the blood-brain barrier, so its effects must be related to actions at some peripheral site. However, there is some evidence that NGF does indeed cross the blood-brain barrier (Kastin et al., 1999; Poduslo and Curran, 1996), so we cannot determine whether peripheral or central actions are responsible for its effects. However, it is relevant that the stimulation of the vagus nerve, essentially a peripheral treatment but with central consequences, has antidepressant-like effects (Nemeroff et al., 2006; Park et al., 2007).

Another consideration is whether the well-known trophic effects of NGF on cholinergic neurons (e.g., Huy et al., 2008; Williams et al., 1991) could be responsible for its antidepressant-like effects. The evidence suggests that this mechanism is more likely to increase immobility, rather than decrease it. Rats with increased cholinergic function tend to have elevated swim test immobility (Overstreet, 2002; Overstreet et al., 1998). Furthermore, there is little evidence that cholinergic antagonists have antidepressant-like effects even though they may increase activity (Overstreet et al., 1995). It is unlikely, therefore, that the trophic effects of NGF on cholinergic neurons contribute to its antidepressant-like effects.

In conclusion, the antidepressant potential for NGF has been established by demonstrating that it reduces swim test immobility in both the FSL rat model of depression and in older outbred Sprague–Dawley rats. However, no effects were seen on c-fos expression, 5-HT_{1A}-mediated hypothermia, fluoxetine-induced increases in corticosterone, and 5-HT_{2A}-mediated head shakes. Thus, NGF has actions predictive of antidepressant efficacy with a novel, as yet undetermined, mechanism of action.

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