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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.

Keywords

Nerve Growth Factor; antidepressants; forced swim test; social interaction test; locomotor activity; desipramine; fluoxetine

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., 2000; 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

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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; 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; Overstreet et al., 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).

Methods

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.

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.

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 grams, so the doses did not differ.

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 hr 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 used also; DMI (5 mg/kg) was injected for 14 consecutive days and the behavioral tests were conducted between 21 and 23 hours 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.

Behavioral Tests—Approximately 20 hr 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, crawling over or under) was recorded during a 5-min session by an experienced observed 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 bottom with its feet. The rat was placed in the swim tank for a single 5-min session 21-23 hr 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).

Statistical Analyses—The data for the three measures in the chronic treatment study were summarized into means±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.

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 et al., 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 two hours 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 hr after the last injection and then sacrificed 2 hr later and the same procedures as for the acutely treated rats were followed.

C-fos expression was assessed by immunochemistry, following the methods developed by Duncan et al., (1996). For all conditions, rats were anesthetized with 70 mg/kg of

pentobarbital sodium two hr. 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 stimului is optimal for immunocytochemical analysis of Fos in brain (Malberg et al., 2000; for review see [Slattery et al., 2005]). Vibratome sections of brain (60 micrometers) 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 (1:20000 dilution; Biognesis, 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 hr, 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 6h 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′-diaminobenzidene 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 select brain regions were counted in a circular field defined by the area of section viewed with a 10 × or 20 × objective, depending of the size of region of interest. In other brain regions, the number of cells within defined histological regions were counted. This approach of cell counting was designed to accurately reflect the number of cells within specific brain regions.

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.

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.

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, DMI treatment).

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 hr 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 hr 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).

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.

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 headshakes were used to assess 5-HT2A 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.

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.

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 hr 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 one hour after the challenge treatments. Chronic treatment continued for two more days and then the swim test was conducted about 22 hr after the last (21st) injection.

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.

Results

1. Chronic NGF Treatment

The results are summarized in Figures 1 & 2 (Swimming), 3 & 4 (Social Interaction) and 5 & 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, Figure 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 completely normalized the behavior of the FSL rats, but NGF did not. Figure 2 shows that, as reported in Figure 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 Figure 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 Figure 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).

The results for locomotor activity, illustrated in Figures 5 & 6, present a different pattern than for swimming and social interaction. Here the standard dose of NGF increased activity of the FSL rats, whereas DMI did not. Still, the activity of the FRL rats was even higher (Fig. 5). As shown in Figure 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 the NGF did increase activity under some conditions could suggestion 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 locomotor activity as the covariate still revealed a highly significant effect on immobility ($F[2,20] = 49.04$, $p < 0.0001$).

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.

3. Blunted Corticosterone Response after Chronic Antidepressant Treatment

The results are summarized in Figure 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 Figure 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.

4. Blunted 5-HT_{1A} and 5-HT_{2A} Responses after Chronic Treatment

The results are summarized in Table 3. In the first column are shown the data for swim test immobility. 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.

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 grams, 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 NFG 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.

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 two hours 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 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} of 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 it effects. However, it is relevant that 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 contributes 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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References

Albert PR, Lembov P, Storring JM, Charest A, Saucier G. The 5-HT_{1A} receptor: signaling, desensitization, and gene transcription. Neuropsychopharmacology 1996;15:213–316. [PubMed: 8840358]

Altar CA. Neurotrophins and depression. Trends Pharmacol 1999;20:59–61.

Angelucci F, Aloe L, Jimenez-Vasquez PA, Mathé AA. Mapping the differences in the brain concentration of BDNF and NGF in an animal model of depression. NeuroReport 2000;11:1369–1373. [PubMed: 10817624]

Angelucci F, Aloe L, Jimenez-Vasquez PA, Mathé AA. Electroconvulsive stimuli alter NGF but not BDNF concentrations in brains of a rat model of depression. Neuropeptides 2003;37:51–56. [PubMed: 12637036]

Angelucci F, Mathe AA, Aloe L. Neurotrophic factors and CNS disorders: findings in rodent models of depression and schizophrenia. Prog Brain Res 2004;146:151–165. [PubMed: 14699963]

Beck CH. Acute treatment with antidepressant drugs selectively increases the expression of c-fos in the rat brain. J psychiatry Neurosci 1995;20:25–32. [PubMed: 7865498]

Benca RM, Overstreet DH, Gilliland MA, Russell D, Bergmann BM, Obermeyer WH. Increased basal REM sleep but no difference in dark induction or light suppression of REM sleep in Flinders Rats with cholinergic supersensitivity. Neuropsychopharmacology 1996;15:45–51. [PubMed: 8797191]

Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 2001;50:260–265. [PubMed: 11522260]

Dahmen N, Fehr C, Reuss S, Hiemke C. Stimulation of immediate early gene expression by desipramine in rat brain. Biol Psychiatry 1997;42:317–323. [PubMed: 9276071]

De Medeiros MA, Carlos Reis L, Eugenio Mello L. Stress-induced c-Fos expression is differentially modulated by dexamethasone, diazepam and imipramine. Neuropsychopharmacology 2005;30:1246–1256. [PubMed: 15714225]

Duman RS. Synaptic plasticity and mood disorders. Mol Psychiatry 2002;7:S29–34. [PubMed: 11986993]

Duncan GE, Knapp DJ, Carson SW, Breese GR. Differential effects of chronic antidepressant treatment on swim stress- and fluoxetine-induced secretion of corticosterone and progesterone. J Pharmacol Exp Ther 1998;285:579–587. [PubMed: 9580601]

Duncan GE, Knapp DJ, Johnson KB, Breese GR. Functional classification of antidepressants based on antagonism of swim stress-induced fos-like immunoreactivity. J Pharmacol Exp Ther 1996;277:1076–1089. [PubMed: 8627519]

File SE, Seth P. A review of 25 years of the social interaction test. Eur J Pharmacol 2003;463:35–53. [PubMed: 12600701]

Gray JA, Roth BL. Paradoxical trafficking and regulation of 5-HT(2A) receptor by agonists and antagonists. Brain Res Bull 2001;56:441–451. [PubMed: 11750789]

Huy CY, Danik M, Manseau F, Trudeau LE, Williams. Chronic exposusre to nerve growth factor increases acetylcholine and glutamate relase from cholinergic neurons of the rat medial septum and diagonal band of Broca via mechanisms mediated by p75NTR. J Neurosci 2008;28:1404–1409. [PubMed: 18256260]

Karege F, Perret G, Bondolfi G, Schwald M, Bertscy G, Autry JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 2002;109:143–148. [PubMed: 11927139]

Kastin AJ, Pan W, Maness LM, Banks WA. Peptides crossing the blood-brain barrier: some unusual observations. Brain Res 1999;848:96–100. [PubMed: 10612701]

Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat brain. J Neurosci 2000;20:9104–9110. [PubMed: 11124987]

Morinobu S, Nibuya M, Duman RS. Chronic antidepressant treatment down-regulates the induction of c-fos mRNA in response to acute stress in rat frontal cortex. Neuropsychopharmacology 1995;12:221–228. [PubMed: 7612155]

Morinobu S, Straussbaugh H, Terwilliger R, Duman RS. Regulation of c-Fos and NGF1-A by antidepressant treatments. Synapse 1997;25:313–320. [PubMed: 9097389]

Nemeroff CB, Mayberg HS, Krahl SE, McNamara J, Frazer A, Henry TR, George MS, Charney DS, Brannan SK. VNS therapy in treatment-resistant depression; clinical evidence and putative neurobiological mechanisms. Neuropsychopharmacology 2006;32:1345–1355. [PubMed: 16641939]

Overstreet DH. The Flinders Sensitive Line rats: A genetic animal model of depression. Neurosci Biobehav Rev 1993;17:51–68. [PubMed: 8455816]

Overstreet DH. Behavioral characteristics of rat lines selected for differential hypothermic responses to cholinergic or serotonergic agonists. Behav Genet 2002;32:335–348. [PubMed: 12405515]

Overstreet DH, Griebel G. Antidepressant-like effects of CRF_1 receptor antagonist SSR125543 in an animal model of depression. Eur J Pharmacol 2004;497:49–53. [PubMed: 15321734]

Overstreet DH, Rezvani AH, Pucilowski O, Gause L, Janowsky DS. Rapid selection for serotonin-1A Sensitivity in rats. Psychiatric Genetics 1994;4:57–62. [PubMed: 8049904]

Overstreet DH, Pucilowski O, Rezvani AH, Janowsky DS. Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of Flinders Sensitive Line rats as an animal model of depression. Psychopharmacology 1995;121:27–37. [PubMed: 8539339]

Overstreet DH, Daws LC, Schiller GD, Orbach J, Janowsky DS. Cholinergic/serotonergic interactions in hypothermia: Implications for rat models of depression. Pharmacol Biochem Behav 1998;59:777–785. [PubMed: 9586831]

Overstreet DH, Knapp DJ, Moy SS, Breese GR. A $5\text{-}HT_{1A}$ agonist and a $5\text{-}HT_{2C}$ antagonist reduce social interaction deficit induced by multiple ethanol withdrawals in rats. Psychopharmacology 2003;167:344–352. [PubMed: 12677355]

Overstreet, DH.; Fredericks, K.; Knapp, DJ.; McMichael, J. The antidepressant-like effects of NGF in a genetic animal model of depression. Presented at meeting of World Federation of Societies for Biological Psychiatry; Sydney, Australia. February 9-13, 2004a;

Overstreet DH, Keeney A, Hogg S. Antidepressant effects of citalopram and CRF receptor antagonist CP-154,526 in a rat model of depression. Eur J Pharmacol 2004b;492:195–201. [PubMed: 15178365]

Overstreet DH, Friedman EF, Mathé AM, Yadid G. The Flinders Sensitive Line rat: A selectively bred putative animal model of depression. Neurosci Biobehav Rev 2005;29(4-5):739–59. Epub 2005 Apr 22. [PubMed: 15925699]

Park MC, Goldman MA, Carpenter LL, Price LH, Friehs GM. Vagus nerve stimulation for depression: rationale, anatomical and physiological basis of efficacy and future prospects. Acta Neurochir 2007;97:407–416.

Poduslo JF, Curran GL. Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. Molec Brain Res 1996;36:280–286. [PubMed: 8965648]

Pucilowski O, Overstreet DH. Effect of chronic antidepressant treatment on responses to apomorphine in selectively bred rat strains. Pharmacol Biochem Behav 1993;32:471–475.

Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, Nakazato M, Watanabe H, Shinoda N, Okada S, Iyo M. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry 2003;54:60–75.

Shirayama Y, Chan AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 2002;22:3251–3261. [PubMed: 11943826]

Shiromani PJ, Overstreet DH, Levy D, Goodrich CA, Campbell SS, Gillin JC. Increased REM sleep in rats selectively bred for cholinergic hyperactivity. Neuropsychopharmacology 1988;1:127–133. [PubMed: 3251494]

Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol Biochem Behav 56:131–137. [PubMed: 8981620]

Slattery DA, Morrow JA, Hudson AL, Hill DR, Nutt DJ, Henry B. Comparison of alterations in c-fos and Egr-1 (zif268) expression throughout the rat brain following acute administration of different classes of antidepressant compounds. Neuropsychopharmacology 2005;30:1278–1287. [PubMed: 15812568]

Stahl S. $5\text{-}HT_{1A}$ receptors and pharmacotherapy. Is serotonin receptor down-regulation linked to the mechanisms of action of antidepressant drugs? Psychopharmacol Bull 1994;30:39–43. [PubMed: 7972628]

Toth M. Peripheral regulation of $5\text{-}HT_{2A}$ receptor. Behav Brain Res 1996;73:183–186. [PubMed: 8788499]

Williams LR, Rylett RJ, Moises HC, Tang AH. Exogenous NGF affects cholinergic transmitter function and Y-maze behavior in aged Fischer 344 male rats. Can J Neurolol Sci 1991;18:403–407.

Zangen A, Overstreet D, Yadid G. High serotonin and 5-hydroxyindoleacetic acid levels in limbic brain regions in a rat model of depression: Normalization by chronic antidepressant treatment. J Neurochem 1997;69:2477–2483. [PubMed: 9375680]

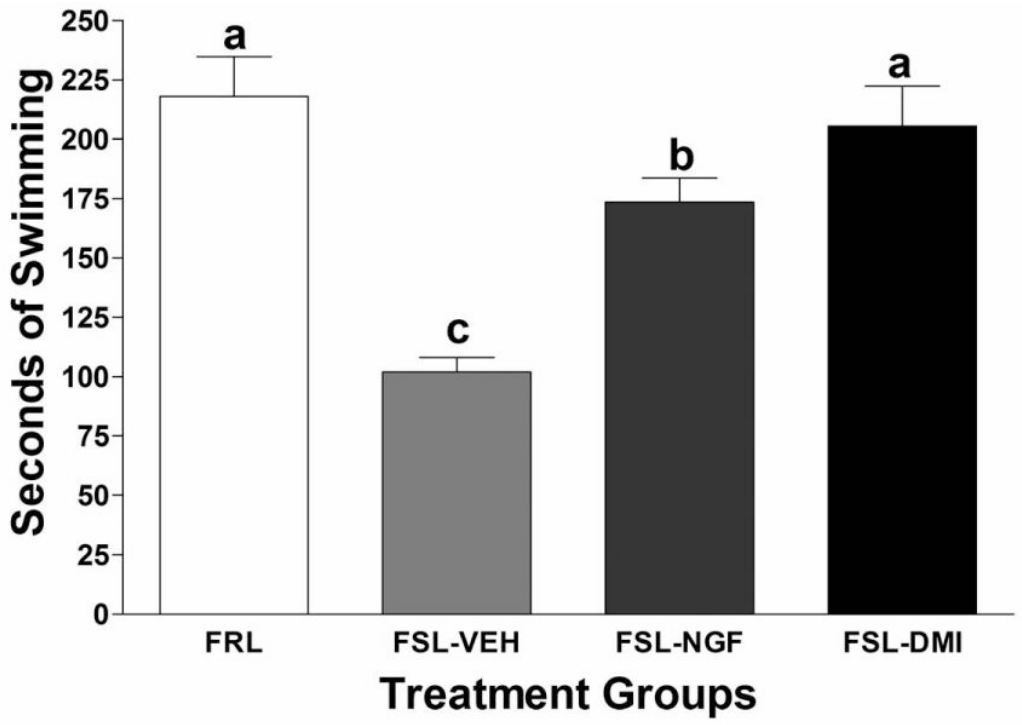

Figure 1. Swimming in FSL & FRL Rats

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 hr after the last treatment. The control FRL rats were given vehicle also. The data represent the mean±s.e.m. Seconds swimming for 8-10 rats.

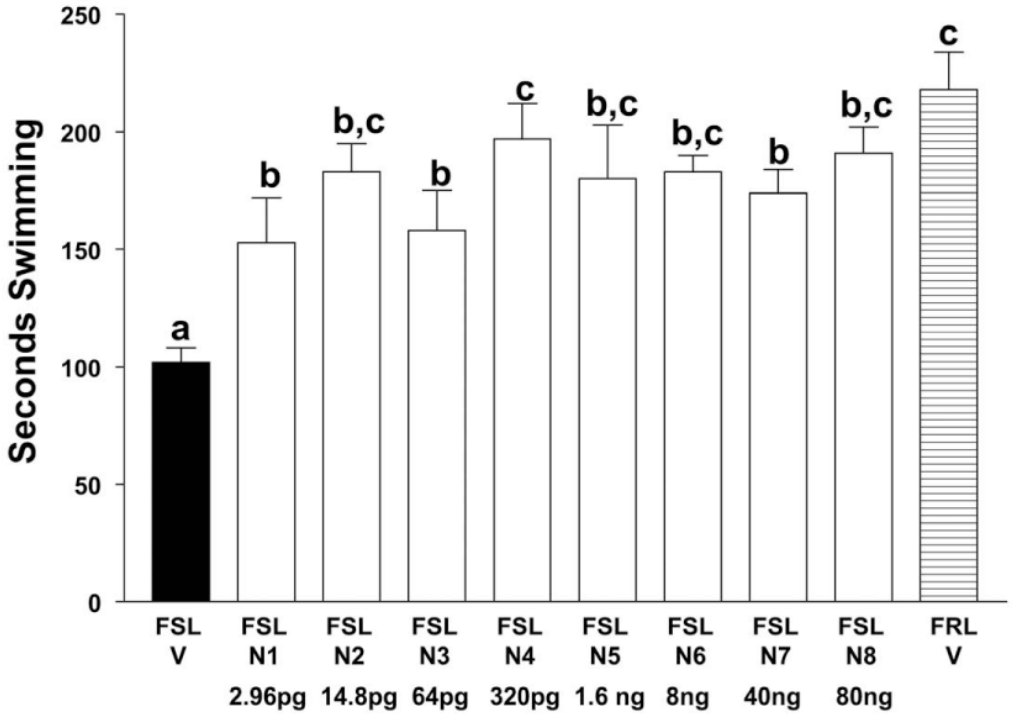

Figure 2. Effects of Different Doses of Nerve Growth Factor on Swimming Time in the Forced Swim Text in FSL Rats

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 hr after the last treatment. The control FRL rats were given vehicle also. The data represent the mean±s.e.m. Seconds swimming for 8-10 rats. Groups with the same letter are not significantly different from each other.

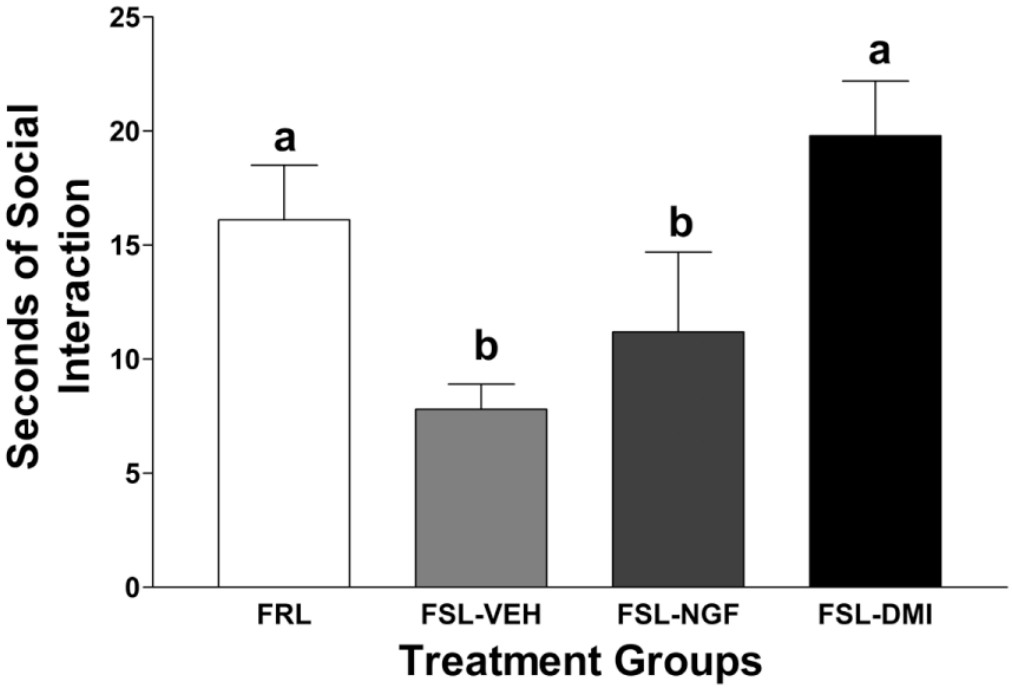

Figure 3. Social Interaction in FSL & FRL Rats

Social Interaction Behavior in FSL Rats after Chronic Treatment with Desipramine (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 hr after the last treatment. The control FRL rats were given vehicle also. The data represent the mean±s.e.m. Seconds of social interaction for 8-10 rats. Groups with different letters are significantly different according to Tukey's tests.

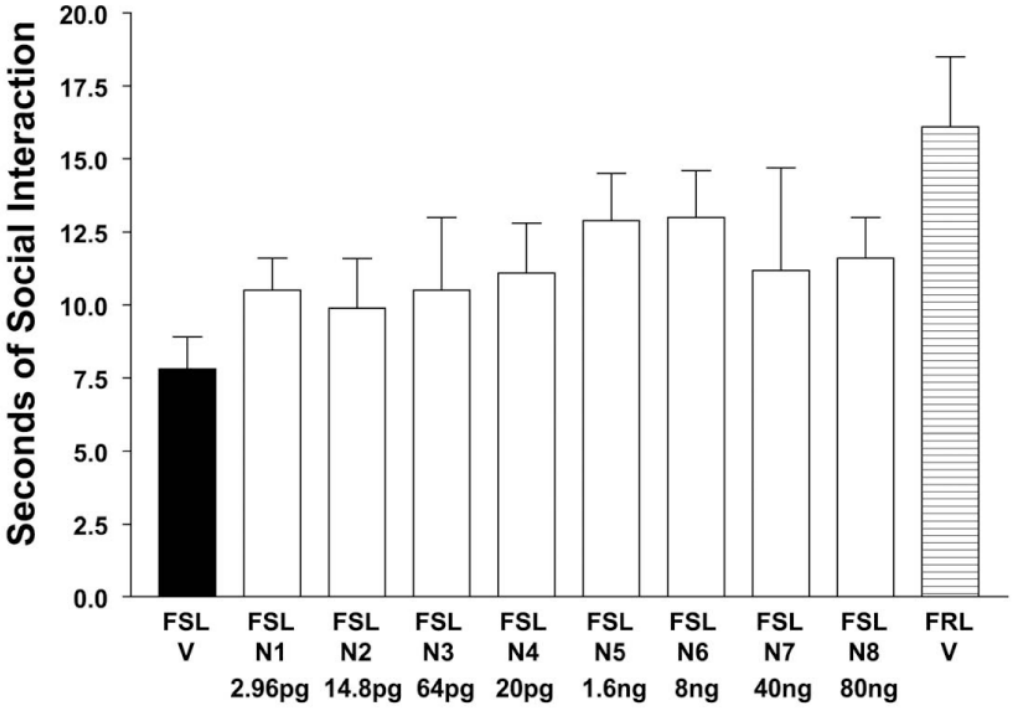

Figure 4. Effects of Different Doses of Nerve Growth Factor on Social Interaction Behavior of FSL Rats

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 hr after the last treatment. The control FRL rats were given vehicle also. The data represent the mean±s.e.m. Seconds of social interaction for 8-10 rats.

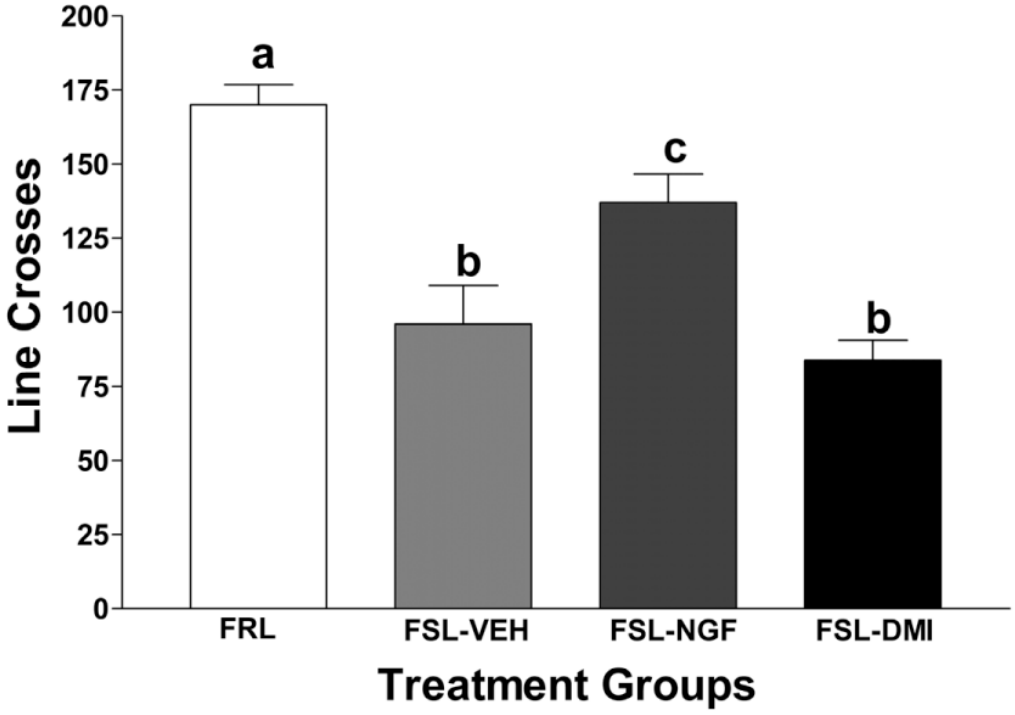

Figure 5. Line Crossings in FSL & FRL Rats

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 hr after the last treatment. The control FRL rats were given vehicle also. The data represent the mean ±s.e.m. lines crossed for 8-10 rats. Groups with different letters are significantly different according to Tukey's tests.

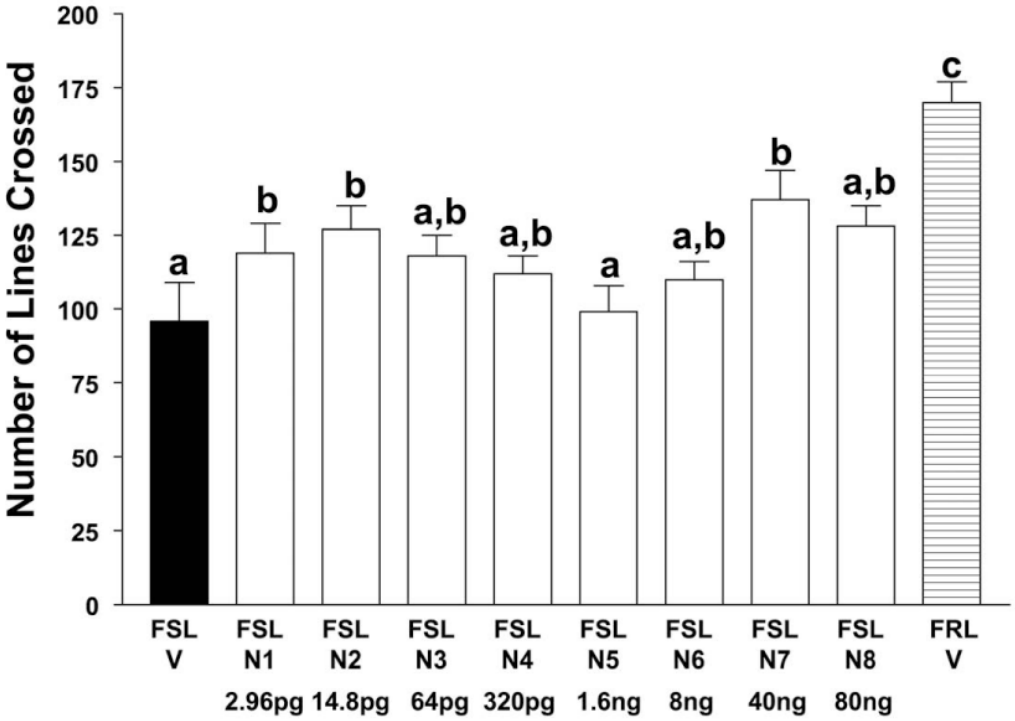

Figure 6. Effects of Different Doses of Nerve Growth Factor on Locomotor Activity of FSL Rats

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 hr after the last treatment. The control FRL rats were given vehicle also. The data represent the mean±s.e.m. lines crossesd for 8-10 rats. Groups with different letters are significantly different according to Tukey's tests.

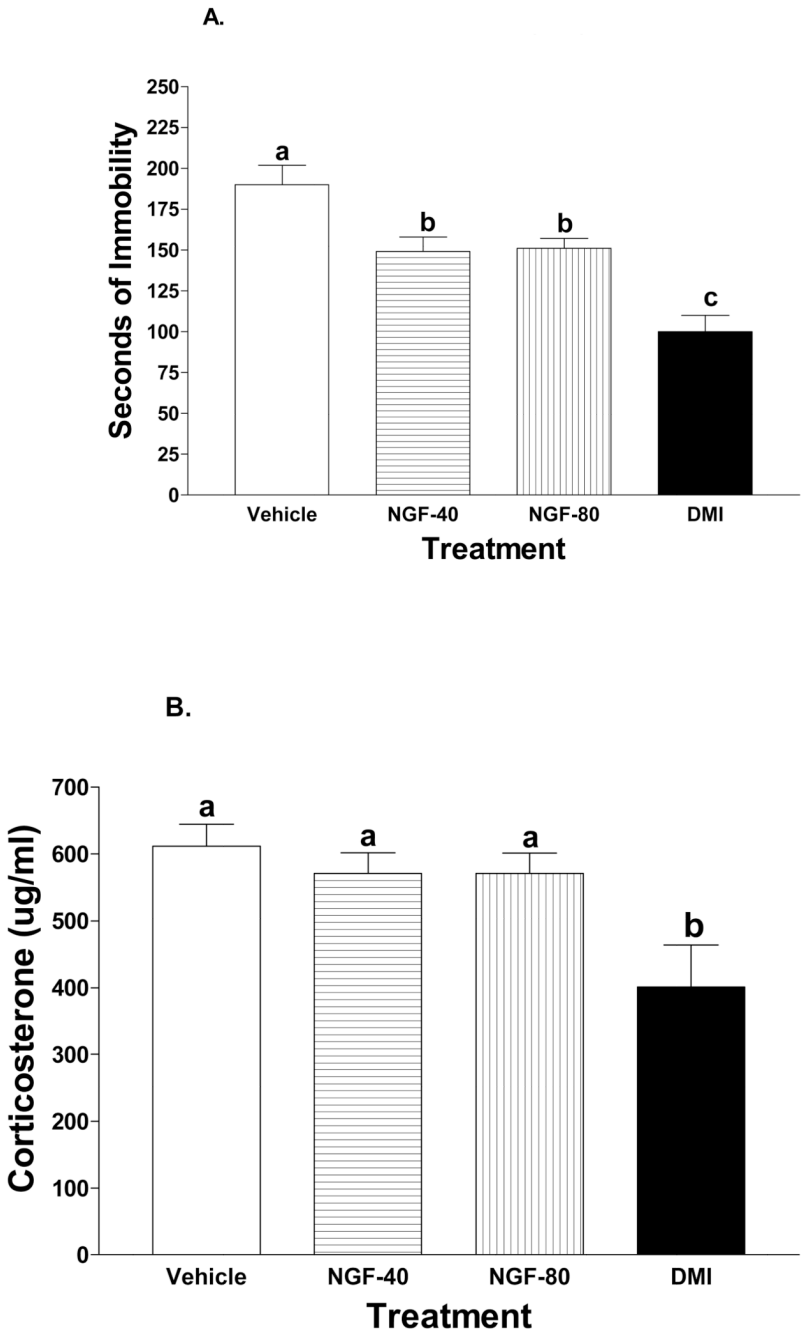

Figure 7.

A. DMI is a More Effective Antidepressant than NGF, but Both Reduce Immobility after Chronic Treatment

B. Only chronic treatment with DMI Reduces Corticosterone Response to Fluoxetine

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 hr 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 hr after the last treatment the

rats were tested in the swim test. The data represent the mean+s.e.m. sec immobile and are presented in the top panel

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±3	13±4	11±3	77±20	79±7	62±8	219±6	166±5*	130±8*

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

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

Table 2

Regional Brain c-Fos Expression after Acute or Chronic Treatment with NGF or Vehicle

Brain Region	Bregma Level^	Grid Used^^	Total Magnification^^^	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*	155± 7*
Central Amygdala	-2.8	10×10	200×	9± 3	5± 1	18± 2*	17± 3*
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*	98±10*
N.Ac. Shell	1.2	5×10	200×	18± 6	12± 7	33± 4*	26± 3*
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
Claustrum	2.7	10×10	200×	53±14	48±22	54±10	59±13

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

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

^^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.

^^^combined objective and eyepiece magnification

Abbreviations: MPC – Medial Prefrontal Cortex, prelimbic subregion; Cx – Cortex; PVN – Paraventricular Nucleus of the Hypothalamus; N. Ac. – Nucleus Accumbens

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*

* $p < 0.05$;

*** $p < 0.001$